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Ammonia inhibition in thermophilic anaerobic process

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Ammonia Inhibition in Thermophilic Anaerobic Process

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Signatures have been redacted for privacy

For my parents: L., Quanming and S., Jiukun

and brother: L., Yang

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CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

Thermophilic anaerobic digestion, operated at a high temperature of 55 °C compared with other treatment processes, offers higher methane production, faster waste degradation, and better pathogen and virus reduction. It was widely used in treating sewage and animal wastes during the past decade. Ammonia is a common hydrolysis product during waste degradation and could cause inhibition when present in high concentrations. This study was conducted to reveal the effect of ammonia inhibition by focusing on methanogenesis, the most susceptible phase in anaerobic digestion. Besides, pH effect was considered, because pH could be an inhibition factor alone and interacts with ammonia inhibition by changing the relative ratio of ammonium to ammonia. Biomass exposed to different concentrations of total ammonia nitrogen (TAN) was used to study the acclimation effect. The response of these microorganisms was examined under inhibitory levels of TAN. In the later part of this study, models for ammonia inhibition and pH effect were assessed.

THESIS ORGANIZATION

There are four chapters included in this thesis. Chapter 1 is a general introduction that gives a background of the research. The following two chapters are two manuscripts detailing different phases of the research project. Chapter 2 is a paper entitled "Effect of Ammonia Inhibition in Thermophilic Anaerobic Digestion". This paper focuses on the

setup of the experiments and the primary results from continuous-flow digesters and batch tests. A simple quadratic regression was used to regress ammonia inhibition with pH effect. Chapter 3 is another paper entitled "Ammonia Inhibition on Thermophilic Aceticlastic Methanogens". This paper describes two models that are feasible for modeling ammonia inhibition and pH effect on methanogenic activity respectively, given the other fixed factor. Chapter 4 summarizes the general conclusions that were drawn from this research.

BACKGROUNDS

Anaerobic Digestion

Anaerobic digestion is a waste treatment process, which completely excludes oxygen from the system. It has been studied intensively in the past decades. In contrast to aerobic process, the organic waste works as both electron donor and electron accepter during anaerobic digestion. Generally, the anaerobic systems are compact and good for treating high strength waste stream. A great advantage of the process is that energy by-product, methane, can be recovered from wastes.

The conversion of complex organic material in anaerobic digestion is a complex process that can be categorized into three stages (Parkin et. al., 1986): 1). Hydrolysis and Liquefaction; 2). Acidogenesis; and 3). Methanogenesis.

Hydrolysis and Liquefaction. Complex organics are degraded in this stage to simple components so that bacteria are able to assimilate them. This process is realized by the extracellular hydrolytic enzyme produced by the bacteria population (Kotze, et. al.,

1969; Parkin et. al., 1986).

Acidogenesis. Organic components are fermented to smaller organic acids and methane precursors, such as propionic, butyric, acetic and valeric acids, and hydrogen.

Methanogenesis. An obligate anaerobe, methanogen, is active in this stage. It uses a few substrates, such as methanol, formic acid and acetic acid, as energy sources. These simple acids are consumed by methanogens for the production of methane. Usually, a sufficient population of methanogens is needed to keep a balanced digestion process.

Thermophilic Anaerobic Digester

An anaerobic digester is a well-mixed tank without solid-liquid separation. It can be treated as a continuous stirred tank reactor (CSTR) operated under anaerobic conditions. It is easy to control the solids retention time (SRT) of the process, because hydraulic retention time (HRT) is identical to SRT. Typically, the SRT in anaerobic digester ranges from 15 to 20 days, depending on the nature of wastes, and longer SRTs are employed if greater waste stabilization is preferred.

Thermophilic anaerobic digestion is operated at a higher temperature of 55 $^{\circ}$C. There are many advantages associated with thermophilic digestion, such as higher methane production, faster throughout, reduced foaming, and near complete destruction of pathogen and virus. The residual solid meets the Class A requirement for land disposal with minimum restrictions.

Inhibition in Anaerobic Digestion

In the maintenance of a stable anaerobic digester, the balance between

methanogenesis and acidogenesis is of importance. Methanogens are more susceptible to high concentrations of toxic compounds (inhibitors). So, the presence of these compounds in anaerobic digestion will disrupt this balance between methanogenesis and acidogenesis. The toxic effects on methanogens will lead to the accumulation of intermediates such as organic acid. The increase in acid concentrations will result in a drop in pH, which further upsets the whole system. This is defined as inhibition.

Anaerobic Toxicity Assay (ATA)

In inhibition study, apart from reactor operation, ATAs are usually run in batches to reveal the bacterial activity and COD reduction rate under different inhibitor concentrations. Soto (1993) reported a modified method for ATA, which was employed in this study. A certain amount of microorganisms, which are of interest for inhibition study, are used in each batch, and usually an excess level of substrate is provided for the batch tests. The substrate is usually substantially higher than K_s (half saturation constant) but not too high to cause substrate inhibition. Since the bacterial activity is near maximum rate under these conditions and independent of substrate concentration, any reduction in the bacterial activity is related to inhibitor's toxicity. To completely evaluate inhibition, ATA needs to run at a series of inhibitor concentrations for a given kind of biomass. In this study, the microorganisms of interest are methanogens, so acetic acid was used as the substrate for the tests. And, the maximum methane production rate in each batch was used as an indicator of methanogenic activity.

Model for Inhibition

The inhibition mechanism on bacterial growth and substrate removal under anaerobic conditions is not well defined and has been receiving attention only recently. Based on Monod kinetics, the model proposed by Han and Levenspiel (1988) is widely accepted. This model quantifies the inhibition effect by adjusting the values of R_m and K_s, as shown in the following equation.

$$R(I)' = R_m\left(1-\frac{I}{I^*}\right)^n\left[\frac{S}{S+K_s\left(1-\frac{I}{I^*}\right)^m}\right]$$

In this equation, $R(I)'$, R_m, S, and K_s are observed microbial activity, maximum microbial activity, substrate concentration, half-saturation coefficient, respectively, same as those defined in Monod equation. I is the inhibitor concentration, while I^* is the lethal inhibitor concentration that can cause microbial activity to totally cease. The magnitudes of n and m determined the type of inhibition. There are four types of inhibitions as defined in Table 1. Identification of the inhibition type is important, because it determines the pattern by which substrate and inhibitor interact in regulating the microbial activity.

Table 1. Summary of inhibition type (Petterson, et. al., 1969)

Inhibition Type	Effect on R_m	n	Effect on K_s	m
Competitive	None	0	Increase	<0
Noncompetitive	Decrease	>0	None	0
Uncompetitive	Decrease	>0	Decrease	>0
Mixed	Decrease	>0	Increase	<0

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CHAPTER 2. EFFECT OF AMMONIA INHIBITION IN THERMOPHILIC ANAEROBIC DIGESTION

A paper to be submitted to the
Journal of Water Environmental Research

Tao Liu and Shihwu Sung

ABSTRACT

This study investigated the inhibition effects of total ammonia nitrogen (TAN) on aceticlastic methanogenic activity in thermophilic anaerobic digestions at various pHs. The thermophilic anaerobic digesters, operated at a solids retention time of seven days and organic loading rate of 4 g COD/L/day, were subject to five different background concentrations of TAN (0.40, 1.20, 3.05, 4.92, and 5.77 g/L). The reactors operated at TAN concentrations of 0.40 g/L and 1.20 g/L showed similar system performance with respect to chemical oxygen demand (COD) removal and methane yield. However, higher TAN concentrations inhibited the digestion process. The TAN concentrations of 4.92 and 5.77 g/L caused 41% and 74% inhibitions on methane production rates. Whereas the digestion presented a case of chronic inhibition, anaerobic toxicity assays (ATA) were conducted to evaluate the acute toxic effect of TAN. The biomass exposed to five different concentrations of TAN was collected from the continuous digesters at steady states and utilized for ATAs. ATAs were run at pHs of 6.5, 7.0, 7.5, and 8.0 and TAN concentrations ranging from 0 to 10.0 g/L, nominally. The results from ATA indicated the inhibition of

aceticlastic methanogens could be affected by pH and acclimation. The highest methanogenic activities were observed at a pH range of 7.0~7.5. The lethal TAN concentration for methanogens was approximately 10.5 g/L, for biomass acclimated to 0.40 and 1.20 g/L of TAN. While, higher acclimation concentrations of 4.92 and 5.77 g/L increased the lethal concentrations to 13 and 14 g/L, respectively.

Keywords:

Anaerobic, thermophilic, ammonium, ammonia, ammonia inhibition, anaerobic toxicity assay (ATA), aceticlastic, methanogenic activity.

INTRODUCTION

Anaerobic digestion at thermophilic temperature offers advantages such as higher pathogen destruction, enhanced hydrolysis of complex biological materials, and significant foaming reduction (Rimkus *et al.*, 1982). It has been the technology of choice for the stabilization of sewage sludge due to better volatile solids destruction, higher biogas production potential and, most importantly, the production of residual biosolids that meet 40 CFR part 503 Class A biosolids specification. Many applications have employed anaerobic digestion processes under thermophilic conditions for the treatment of animal wastes (Skjelhaugen, 1998) and organic wastes. Although anaerobic digestion at thermophilic temperatures has several advantages, it is common knowledge that operation at higher temperatures is sensitive to environmental changes. Even slight variation in operational parameters such as pH (Visser *et al.,* 1993), temperature (Van Lier *et al.,* 1996),

etc. can disturb the process. It is in this context that the inhibition effect of ammonia on thermophilic anaerobic process becomes significant.

Ammonia is the main hydrolysis product produced during the degradation of organic proteineous material. Thermophilic anaerobic digesters used for the treatment of animal wastes such as swine manure (Hansen *et al.*, 1997), cattle wastes (Santha and Sung, 2001) and some of the industrial wastes such as food processing waste streams often encounter very high concentrations of ammonia. Ammonia is an essential nutrient for anaerobic microbes. Total ammonia nitrogen (TAN) concentrations of approximately 200 mg/L (McCarty, 1964) are reportedly beneficial for the anaerobic process. However, higher concentrations of TAN could decrease microbial activities. Many studies (Van Velsen, 1979; Melbinger and Donnellon, 1971) observed toxic effects of ammonia at TAN levels as high as 2,000 mg/L.

Free ammonia was thought to be the primary cause of inhibition since it could penetrate the microbial cell wall easier than the charged ammonium ion (Koster and Koomen, 1988; Zeeman *et al.*, 1985). However, Lay *et al.* (1998) found that concentration of ammonium ion (NH_4^+) was a more significant factor than free ammonia in the determination of thermophilic methanogenic activity. It was also found that acclimation to ammonia could increase the tolerance of methanogens to high TAN levels. Mesophilic system studied by Parkin (1982) performed well at 9,000 mg/L of TAN after acclimation.

In anaerobic systems, equilibriums exist among ammonium ion ($NH_4^+{}_{(aq)}$), free ammonia ($NH_{3\,(aq)}$) in solution, ammonia ($NH_{3\,(g)}$) in gas phase, hydrogen ion (H^+) and hydroxyl ion (OH^-). These equilibriums are described in Equations 1, 2 and 3:

$NH_4^+{}_{(aq)} \Leftrightarrow NH_{3\,(aq)} + H^+$ $K_a = [NH_{3(g)}][H^+]/[NH_4^+{}_{(aq)}]$ (1)

$H_2O \Leftrightarrow H^+ + OH^-$ $K_w = [H^+][OH^-]$ (2)

$NH_{3\,(g)} \Leftrightarrow NH_{3\,(aq)}$ $K_H = [NH_{3(aq)}]/[NH_{3(g)}]$ (3)

Where

K_a, K_w, K_H = equilibrium constants.

The above equations are temperature dependent. Based on Van't Hoff relationship, the following equations can be derived from Eqs. 1, 2, and 3:

$K_a = \exp(-6250.6/T - 0.3537)$ (4)

$K_w = \exp(-6723.9/T - 9.6766)$ (5)

$K_H = \exp(4167.1/T - 18.0265)$ (6)

Where

T = temperature (°K).

Table 1. Constants related to ammonia at different temperatures

Temperature (°C)	pK_a	pK_w	pK_H
25	9.26	14	1.76
35	8.97	13.7	1.95
55	8.43	13.1	2.31

Table 1 lists the equilibrium constants (pK_a, pK_w and pK_H) calculated from Eqs. 4, 5, and 6 at various temperatures. These equations and constants show that pH and temperature are critical parameters that could affect the pattern of ammonia inhibition, since pH and temperature can change the [ammonium]/[ammonia] ratio. Ammonia inhibition is actually the combined effect of ammonia, ammonium and pH at a given temperature, which should be studied collectively.

MATERIALS

Anaerobic Digestion Systems

Two completely stirred tank reactors (CSTRs) were used as anaerobic digesters in this study. They were cylindrical tanks made of plexiglass with an inside diameter of 10 inches, an outside diameter of 11 inches, and a depth of 14 inches. The total volume of each digester was approximately 16 liters with an active volume of 14 liters. Four vertical baffles 0.25-inch width were evenly spaced along the height of each reactor to ensure complete mixing of the digester contents. The digesters had five ports at the top for mixer shaft, feed, decant, biogas, and pH meter. Hot water bath was provided for the digesters to maintain the temperature at 55±1°C. Reactor feeding and decanting was carried out by Masterflex tubing pumps (Model: 7553-50), and mixing by an Eastern Mixer (EMI, Model: pm6015). The gas collection system for each of the reactors consisted of five components: (1) a gas reservoir for gas equalization, (2) a gas observation tube, (3) a gas sampling port for gas sampling, (4) a H_2S scrubber (steel wool), and (5) a rotary drum wet-tip gas meter for recording the daily gas production. Figure 1 shows the setup of the anaerobic digesters.

The two digesters were operated at thermophilic conditions (55 ±1°C) and were fed with soluble non-fat dry milk as organic substrate throughout the course of the study. The characteristics of non-fat dry milk are listed in Table 2. Both digesters were operated at a COD loading rate of 4.0 g/L/day and a hydraulic retention time (HRT) of 7 days. The study was conducted in five phases. During the first phase, the background TAN concentration in the feed to the digester was approximately 0.4 g/L and no additional ammonia was added. For the following phases, ammonium chloride was added to increase the TAN concentration to the desired levels. Each TAN concentration was maintained until the digesters reached quasi-steady states, which were defined by constant daily biogas production, effluent volatile fatty acid (VFA) concentrations and operating pH values within 5% variance for more than five consecutive days in each reactor. Usually, steady state conditions were attained after 30-35 days (5 HRTs) of operation. The biomass collected at steady state was used as acclimated biomass for ATA. The TAN concentrations inside the reactor during the five phases were reported to as the "acclimation concentration" in this paper.

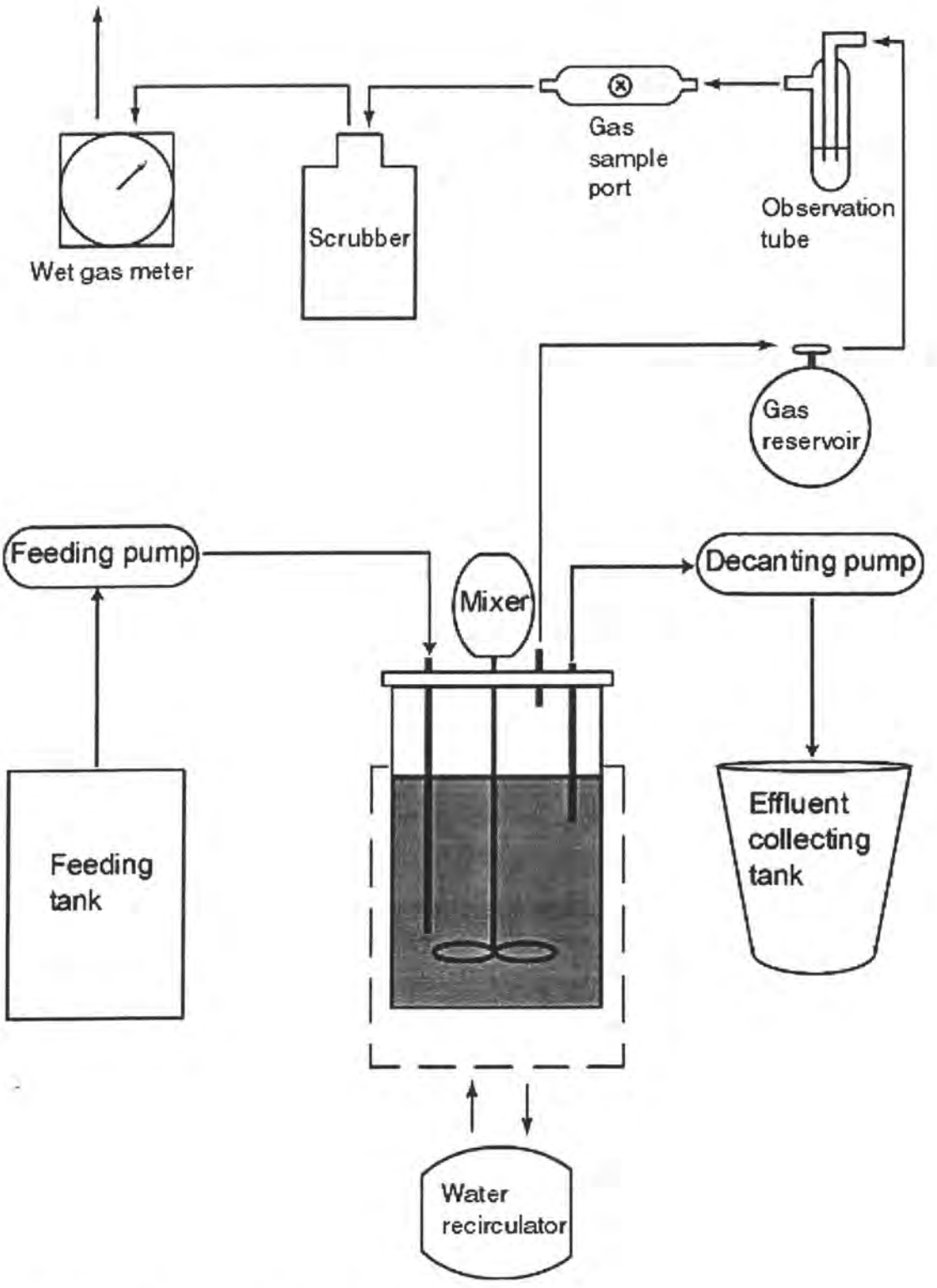

Figure 1. Anaerobic digester system setup

Table 2. Characteristics of non-fat dry milk[a]

Chemical oxygen demand, g/g NFDM	1.03
Five-day biochemical oxygen demand, g/g NFDM	0.49
Total organic carbon, g/g NFDM	0.21
Total Kjeldahl nitrogen, % as N	5.4
Total phosphate, % as PO_4	2.2
Lactose, g/100g NFDM	51.0
Protein, g/100g NFDM	>36.0
Fat, g/100g NFDM	<1.0
Ash, g/100g NFDM	8.2

[a] Manufactured by Kraft General Foods, Inc.

Anaerobic Toxicity Assay (ATA)

The acclimated biomass collected from the two digesters was used in batch anaerobic toxicity assays, which were conducted in 250 ml serum vials containing 100 ml of mixed liquor of acclimated biomass from the digesters. Acetic acid (HAc) was used in batch tests as main substrate to study aceticlastic methanogenic activity. The vials were sealed with butyl rubber stoppers and incubated in a shaker chamber set at 200 rpm and 55°C. For each set of experiments, pH was buffered and TAN concentration was controlled at a predetermined level. Each experiment was conducted in duplicates. Biogas production was measured at intervals of 4-12 hours. Cumulative methane production was then calculated over time. The maximum methane production rates divided by the amount of volatile suspended solids were used as specific methanogenic activities (SMAs) for inhibition investigation.

Sampling and Analysis Methods

Alkalinity (ALK), volatile suspended solids (VSS), volatile fatty acid (VFA), and chemical oxygen demand (COD) were measured according to the Standard Methods (American Public Health Association, 1980). The pH and biogas production were recorded daily. All pH values were measured by an Orion pH probe. Biogas production was measured using a wet-tip Gas Meter (Rebel Point Wet Tip Meter Co.). The gas production in batch tests was measured using glass syringes of different volumes equipped with metal hub needles. The methane in biogas (CH_4 %) was measured using a GOW-MAG series 350 gas chromatography (GC) with Porpak Q 80/100 mesh column. The

operating temperatures of the injection port, the column, and the detector were 160, around 70, and 200 °C. Helium was used as the carrier gas at 40 psi. The GC system was calibrated using a custom-made gas standard that contained 5% nitrogen, 70% methane, and 25% carbon dioxide. The minimum detectable amount of methane was 1%.

METHODS

Modeling Batch Methane Production

For ATA, the biogas production was recorded over time. Generally, the biogas production rate shows a trend where it picks up from zero and accelerates to a maximum (R'), after an initial lag phase. With time, the biogas production rate drops back to a minimum value. The total amount of methane produced is called methane production potential (P). A number of models can been used to describe this process, which include Gompertz, Stannard et. al.(1985), Gibson et.al. (1987), Schnute(1981), and logistic model(1979). Zwietering et. al. (1990) compared these models based on the lack of fit test with measuring error. The modified Gompertz equation (Eq. 7) has been proved statistically to be sufficient to describe the bacterial growth. Based on the study of Lay et. al. (1996), who related the bacterial growth to metabolic biogas production, the Gompertz equation was employed in this study to describe the cumulative methane production curve in the batch experiments (ATA).

$$M = P \cdot \exp\{-\exp\left[\frac{R' \cdot e}{P}(\lambda - t) + 1\right]\} \qquad (7)$$

Where

M = cumulative methane production (ml CH_4);

λ = lag-phase time (day);

P = methane production potential (ml CH_4/bath);

R' = methane production rate at a pH and a inhibitor concentration (ml CH_4/day);

e = constant (2.71828).

The parameters (P, λ, and R') in the above non-linear model were estimated using least square estimation (Gauss-Newton procedure). A Taylor expansion was used to approximate the above model with a linear function of the parameters. Reasonable starting values were taken for estimates of the parameters. Then, ordinary least square (OLS) estimation was applied to the linear approximation to "update" the estimates of parameters. The iterations (Eq. 8) were therefore continued until some convergence criterion was satisfied:

$$SSE^{(i-1)}-SSE^{(i)} < \text{a constant} \qquad (8)$$

Where

SSE = sum square of error.

Since the research focus was the inhibition effects on methanogens, acetic acid was used as the sole substrate in batch tests. It was assumed that only methanogenesis occurred in the batch tests without the acidogenic phase. It was also assumed that no significant difference in methane production potentials (P's) from the batches existed using the same acclimated biomass. However, different pH and TAN concentrations resulted in different R', the maximum methane production rates. The parameter R' was used as a major

indicator for ammonia inhibition.

Inhibition Models and Quadratic Regression

The Monod equation has been widely applied to describe the growth kinetics of bacteria. However, it was doubtful whether the use of Monod equation could describe the growth kinetics of bacteria in the presence of inhibitors. In order to solve this problem, Han and Levenspiel (1988) proposed an extended Monod equation, as follows:

$$R(I)' = R_m(1-\frac{I}{I^*})^n\left[\frac{S}{S+K_s(1-\frac{I}{I^*})^m}\right] \quad (9)$$

where

R(I)' = methane production rate observed a inhibitor level (ml CH_4/g VSS/day);

R_m = maximum methane production rate (ml CH_4/g VSS/day);

S = substrate concentration (mg COD/L);

K_s = half-saturation coefficient (mg COD/L);

I = inhibitor concentration (g/L);

I^* = lethal inhibitor concentration (g/L);

n, m = coefficients.

The parameter R' in the Gompertz equation (Eq. 7) was determined at various pHs and inhibitor concentrations in ATA and was used as R(I)' in the above extended Monod equation. I is the corresponding inhibitor concentration for each R'.

The extended Monod equation includes three extra parameters (n, m, and I^*) to

embody the inhibition effects. Based on the studies of Lay et. al. (1998), the above equation could be reduced to a simplified form, $R(I)=R_m(1-I/I^*)^n$, which only included two parameters for inhibition effects. The simplified equation was easier to use, since it could be transformed to a linear model if lethal inhibition concentration was given. However, in ammonia inhibition, inhibitors include ammonia, ammonium ion, hydrogen ion, and hydroxyl ion. The combined inhibition effect would be hard to explain by extended Monod equation or simplified form, since they are generally used for single inhibitor.

In order to establish the relationship between inhibition response and explanatory values such as ammonia, ammonium, hydrogen ion, etc., quadratic regression model (Box, et. al., 1978; Sen and Srivastava, 1990; Fox, 1997), a second-order multi-linear regression, was used. The quadratic regression is defined as follows:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1^2+\beta_4X_2^2+\beta_5X_1X_2+\varepsilon \qquad (10)$$

Where

Y = dependent variable;

X_1, X_2 = independent variables (predictors);

β_0, β_1, β_2, β_3, β_4, β_5 = coefficients;

ε = error term.

In the above equation, explanatory variables can be more than two. To simplify the model and determine the independent variables more precisely, a variable selecting procedure was necessary. The backward elimination procedures proposed by Sen and Srivastava (1990) was employed to reduce the explanatory variables such as $[NH_3]$, $[NH_4^+]$, $[H^+]$, $[OH^-]$, [TAN], pH, etc. to two variables, TAN and pH. Based on Eqs.1 and 2,

$[TAN]=[NH_3]+[NH_4^+]$, and $pH=-\log([H^+])$, these two parameters are independent and therefore could used in the above quadratic regression.

RESULTS AND DISCUSSIONS

Performance of Anaerobic Digesters Operated at Different Levels of TAN Concentrations

In this study, the nominal TAN concentrations of the feed prepared for the digesters were 0, 1.0, 3.0, 5.0, and 6.0 g/L through five phases of operation. As shown in the Table 3, the resulting TAN concentrations at steady states were slightly different from the nominal concentrations. This actual TAN concentration was the major factor that contributed to the difference among the system operations, since all the other operation parameters were identical. By subjecting the digesters to gradually increasing levels of TAN, an attempt was made to simulate a chronic toxicity condition. Under these conditions, the methanogens were allowed sufficient time to adapt to the higher concentration of TAN and retain the SMA. The acclimation TAN concentration was used to label the biomass collected at each operating condition.

The overall performance of anaerobic digesters at each phase is shown in Figure 2 with more measurements summarized in Table 3. At higher TAN concentrations, the digesters became unstable and it took longer time to attain a steady state. As seen from Figure 2, decrease in methane production rates at higher TAN concentration indicated the inhibition of methanogens. The methane production rates revealed the activities of

acclimated methanogens at the specific acclimation condition. At acclimation concentrations of 4.92 and 5.77 g/L, methane production rates dropped approximately by 41% and 74%, respectively, compared with the methane production rates during the first phase. This matched the drop in COD removal rates. At the same time, as TAN concentration increased from 3.05 g/L to 5.77 g/L, a drop in pH was also observed. This suggested the accumulation of organic acids inside the digesters, as indicated by an increase in VFA and decrease in ALK.

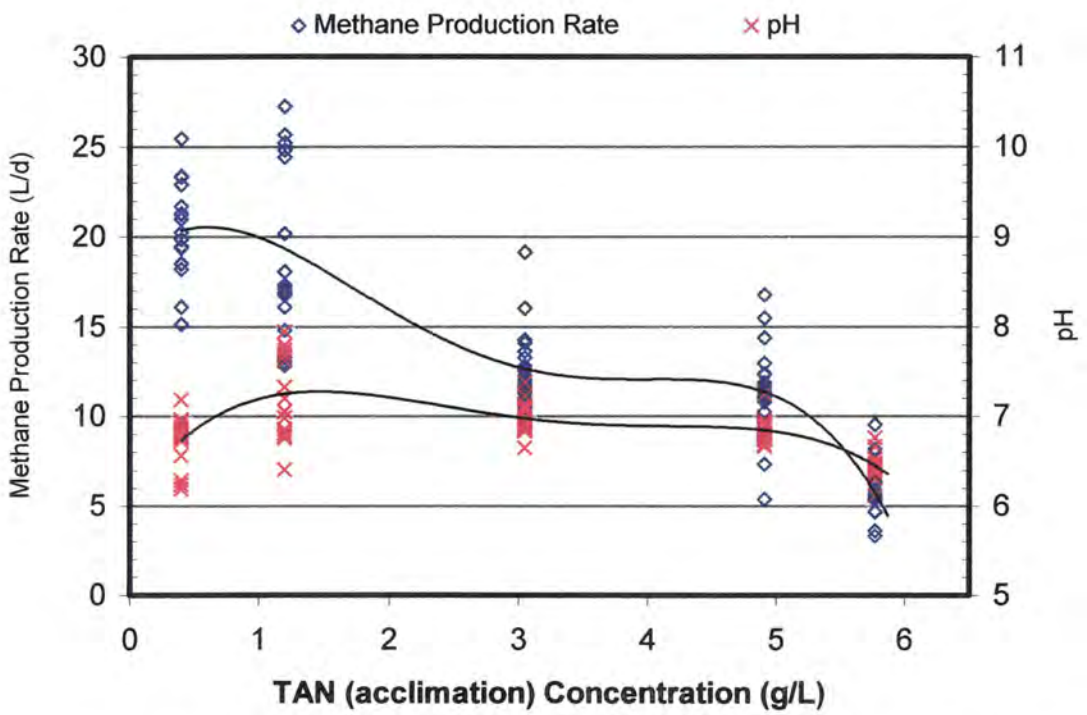

Figure 2. Methane production rates and pHs during five phases of anaerobic digester operation

Table 3. System performance of anaerobic digesters under various TAN loading rates

Nominal TAN Loading (g/L)	TAN Concentration inside Reactor (g/L)	MLVSS (g/L)	VFA (mg/L as HAC)	ALK (mg/L as $CaCO_3$)	COD Removal Rate (%)
0	0.40	1.43	2,034	3,101	75
1.0	1.20	1.53	2,213	3,384	78
3.0	3.05	2.10	1,923	3,670	72.3
5.0	4.92	2.03	2,553	3,185	62.6
6.0	5.77	1.96	2,733	3,027	49.9

Monod Constants Determination

A series of serum bottle toxicity assays (ATA) were run at different substrate (acetic acid) concentrations (110.9 mg/L, 537.6 mg/L, 964.3 mg/L, 1,390.9 mg/L, and 1,817.6 as COD) using biomass from the digester in operation during the first phase. The background TAN concentration was 0.40 g/L and no ammonium chloride was added. It was assumed that under this condition, there was no ammonia inhibition and a set of baseline kinetic constants of aceticlastic methanogens was determined.

For each batch, the methane production was measured at regular intervals of time. The cumulative methane production is plotted (markers) in Figure 3 and regressed by Gompertz equation using non-linear least square regression (Eq.7). The simulated results from Gompertz equation are shown as smooth curves. Monod kinetic constants were subsequently determined by linear regression for the transformed Monod equation to be K_s=694.4 mg/L as COD and R_{max}= 1,927 ml CH_4/g VSS/day.

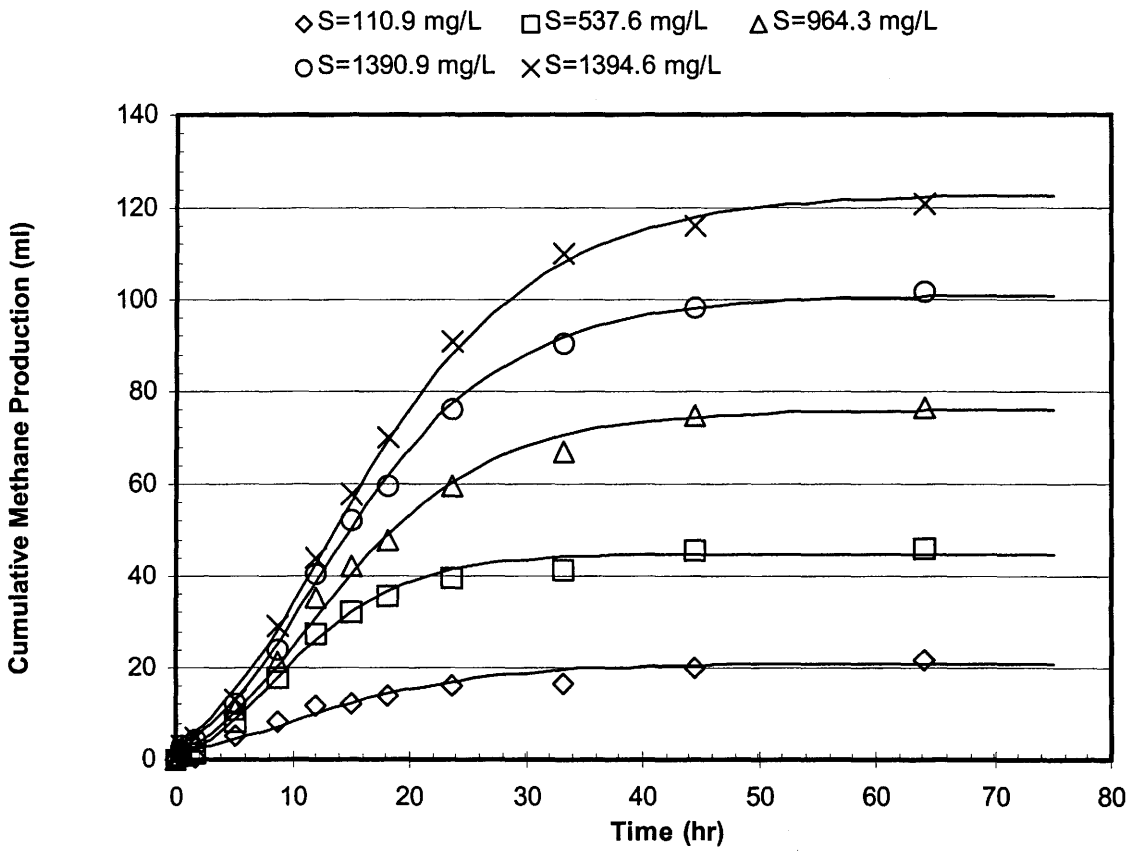

Figure 3. Cumulative methane production at different initial substrate (S) concentrations as COD

Specific Methanogenic Activities determined from Anaerobic Toxicity Assay (ATA)

To study acute toxicity response of TAN in batch experiments, a fixed substrate concentration of 1,800 mg/L as COD and different dosages of ammonium chloride were added to the batch bottles. The batch tests measured the initial response of methanogens to the high dosage of TAN. This minimized the adaptation time for methanogens and caused an acute toxic condition with little time to recuperate. It was assumed that at the substrate level in batch tests, there was no substrate limiting condition or substrate inhibition.

The cumulative methane production curves in Figure 4 show a typical set of results from ATA batch experiments. For this set of ATA results, the acclimated biomass used was collected from the digester with an acclimation concentration of 0.40 g/L. This set was run at a pH of 7.0 and ammonium chloride was added to all but the control batches to increase TAN to predetermined nominal concentrations. The actual TAN concentration in each batch was corrected to include the TAN concentration in the biomass seed, and calculated as nominal [TAN] +acclimation [TAN] · dilution rate.

As shown in Figure 4, lag phases were not significant and the batches had similar methane production potentials (Ps) of approximately 120 ml CH_4. The differences in slope during exponential methane production phases for these curves showed the inhibition effects of TAN at pH 7.0. The modified Gompertz equation (Eq.7) was used to fit the cumulative methane production, predicted values as shown by solid lines in Figure 4.

The batch experiments were repeated for pH 6.5, 7.0, 7.5, and 8.0, and the regression results of each estimated parameter are listed in Table 4. The regression results

and estimates of the parameters for acclimation concentrations of 1.20, 3.05, 4.92, and 5.77 g/L are summarized in Tables 5, 6, 7, and 8, respectively.

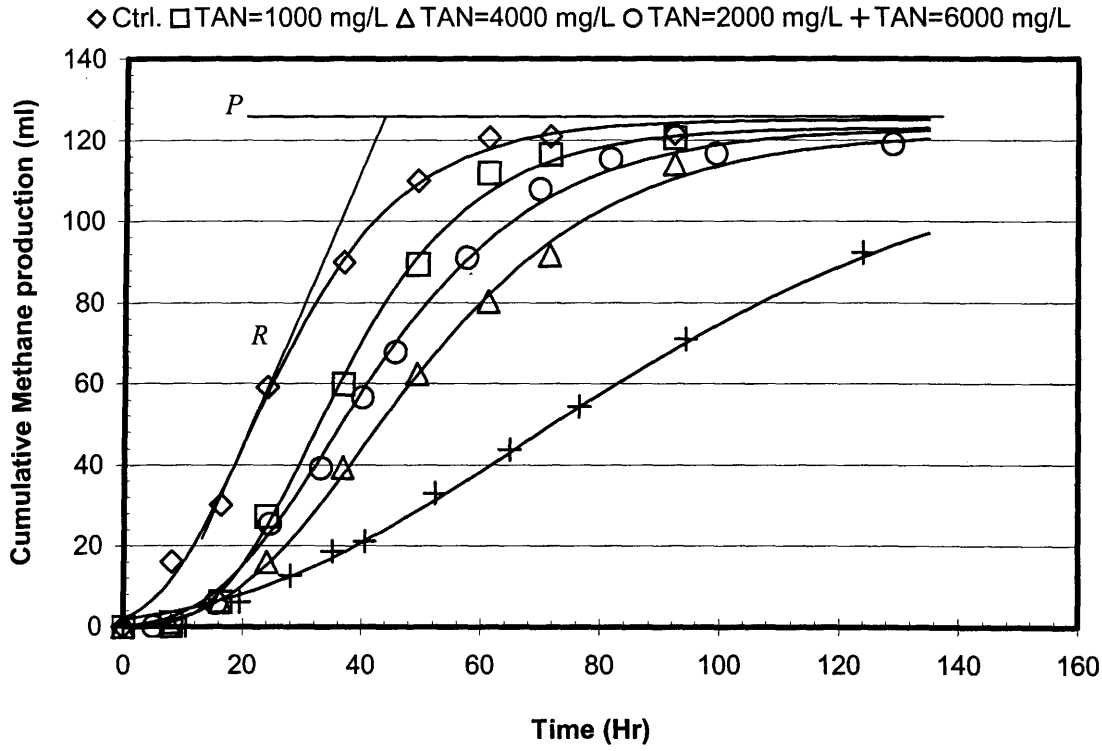

Figure 4. Cumulative methane production curves in batch experiments at pH 7.0 with acclimated biomass obtained in the first phase

Table 4. Parameters determined from ATA with biomass acclimated to a TAN concentration of 0.40 g/L

Additional	pH = 6.5					pH = 7.0					pH = 7.5					pH = 8.0				
TAN conc.	P[a]	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2
Ctrl.	125	740	1	176	.98	111	1104	6	105	.99	115	1472	.4	70	1.0	140	1120	2.5	90.3	1.0
	129	756	2	251	.98	138	1174	8	240	.99										
1.0	116	781	2	265	.98	120	973	16	428	.98	121	1504	.7	46	1.0					
	124	667	3	308	.98	127	992	17	183	.99										
2.0	131	635	9	197	.99	123	746	15	30	1.0	119	1408	.9	55	1.0	161	994	3.9	191	.99
	124	748	6	207	.99	124	749	16	49	1.0						140	979	3.9	93	1.0
3.0	110	660	3	122	.99						119	1280	.8	64	1.0					
4.0	124	488	15	122	.99	122	637	18	48	1.0	121	1168	.9	57	1.0	149	748	5.5	144	.99
	125	472	9	118	.98	122	640	18	46	1.0						142	742	4.6	144	.99
5.0	112	423	5	165	.98						118	960	.7	79	1.0	135	661	7	84	1.0
	94	390	1	159	.97											138	661	6.5	105	.99
6.0						98	368	20	19	.99	119	864	.6	84	1.0	113	499	5	77	.99
7.0											114	781	.7	110	.99	96	430	5	67	.99
																87	440	3.4	78	.99
8.0	83	277	6	57.6	.98											75	385	3.2	105	.98
																81	387	2.3	56	.99
9.0																73	343	3.4	39	1.0
																75	352	2.2	59	.99
10.0																18	68.6	4	3.7	1.0
																28	159	5	3.3	1.0

Units of Additional TAN concentration, P, R' and λ are g/L, ml CH_4, ml CH_4/g VSS/day and hr, respectively.

Table 5. Parameters determined from ATA with biomass acclimated to a TAN concentration of 1.20 g/L

Additional	pH = 6.5					pH = 7.0					pH = 7.5					pH = 8.0				
TAN conc.	P[a]	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2
Ctrl.	112	803	2.5	211	.98	102	1136	3.2	95.5	.99	118	1391	5	78	1.0	124	1196	2	85.2	1.0
											113	1369	4.7	62.8	1.0					
2.0	113	803	2.5	175	.99	97	1180	3.3	58.7	1.0	121	1401	3.9	68.1	1.0	119	1198	4.6	126	.99
	114	777	5	104	.99	112	1162	4	47.7	1.0	124	1409	4.5	46.9	1.0	121	1238	3.5	76.2	1.0
4.0	124	750	13	15.0	1.0	106	1018	5	26.6	1.0	116	1315	3.8	54.2	1.0	116	1030	6	100	.99
	117	669	13	3.42	1.0	82	1004	4	111	.98	113	1273	4	61.3	1.0	105	1082	6.5	106	.99
5.0	124	482	12	22	1.0	107	1029	4	76.6	.99	110	1260	5	101	.99	107	938	11	95.2	.99
	119	616	15	2.15	1.0	106	852	4	145	.99	112	1226	5.2	95.6	.99	95	872	8	105	.99
6.0	111	402	6	87.7	1.0	100	773	3.9	72.8	1.0	106	997	5.6	91.2	1.0	94	822	6	90.2	1.0
	112	295	15	34.3	1.0	107	753	5	118	.99	104	1071	5.4	106	.98					
7.0	121	180	10	104	.99	58	461	4	30.5	.99	98	943	7.5	112	.99	71	589	2.3	85.1	.99
	110	194	10	14.6	1.0	85	390	8	101	.98	88	977	6.5	87.6	.98	65	586	4.6	94.2	.98
8.0	5	27	10	6.82	.72	75	426	3.2	105	.98	78	725	10	84.6	.98	66	506	10	86.2	.98
	46	134	9	31.2	.98	20	142	2	22.5	.94	79	675	12	73.5	.98	49	546	12	65.2	.98
9.0	5	27	9	8.32	.54	11	35	2	5.0	.93	69	555	11	94.5	.98	46	339	12	100	.96
	6.5	27	9	8.27	.74	18	71	4	4.2	.99	71	555	9	131	.99	51	350	13	146	.96
10.0						20	38	4	64.8	.81	58	288	7.8	106	.97	20	130	16	106	.98
						4.5	10	4	6.1	.56	39	315	12	52	.93					

Units of Additional TAN concentration, P, R' and λ are g/L, ml CH_4, ml CH_4/g VSS/day and hr, respectively.

Table 6. Parameters determined from ATA with biomass acclimated to a TAN concentration of 3.05 g/L

Additional TAN conc.	pH = 6.5					pH = 7.0					pH = 7.5					pH = 8.0				
	P[a]	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2
Ctrl.	118	765	3.7	189	.99	127	968	5.0	89.4	.98	108	1030	4.7	54.6	1.0	118	718	6	98.1	1.0
2.0	121	765	3.5	129	.99	131	970	2.9	77.8	1.0	135	1055	5.6	108	1.0	121	721	5.7	94	1.0
	125	776	4.0	86.1	1.0	120	976	4.2	67.2	1.0	133	1070	3.4	117	1.0	125	741	4.9	104	1.0
4.0	120	577	18	123	.99	117	791	8.1	102	1.0	125	959	9.4	119	1.0	108	571	7.1	127	.99
	117	561	16	187	.99	116	801	7.2	59.6	1.0	119	945	5.8	128	1.0	112	579	8.6	102	1.0
6.0	115	431	15	125	.99	120	573	5.6	123	.99	123	604	11	89	1.0	104	396	11	124	.94
	118	419	17	84.5	.99	112	568	13	149	.99	127	628	9.1	103	.98	99	378	14	123	.94
8.0	101	151	13	201	.98	107	308	19	105	.99	108	392	12	132	.98	74	196	17	107	.92
	97	155	19	174	.99	98	258	12	126	.98	107	334	16	109	.99	68	178	21	84.1	.98
10.0	55	43	22	123	.87	60	39.1	31	77.2	.84	98	58	14	101	.91	17	22	23	94	.88
	39	37	21	164	.68	49	41.7	19	132	.80	94	57	18	79.2	.93	40	38	21	78.1	.75

Units of Additional TAN concentration, P, R' and λ are g/L, ml CH_4, ml CH_4/g VSS/day and hr, respectively.

Table 7. Parameters determined from ATA with biomass acclimated to a TAN concentration of 4.92 g/L

Additional	pH = 6.5					pH = 7.0					pH = 7.5					pH = 8.0				
TAN conc.	P[a]	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2
Ctrl.	133	502	3.1	135	.99	126	612	2.5	126	.99	130	579	3.4	115	.99	123	518	3.2	98	.99
2.0	122	514	4.1	103	.99	119	627	2.5	133	.99	128	613	2.9	103	.99	120	537	4.5	128	.99
	125	520	4.6	105	.99	124	629	3.0	124	.99	132	609	4.3	114	.99	125	539	4.4	114	.99
4.0	121	477	8.6	138	.99	106	626	6.2	148	.99	127	586	4.1	123	.99	112	520	8	124	.99
	118	479	8.4	146	.99	121	622	6.3	121	.99	122	588	8.1	135	.99	110	514	7.4	119	.99
6.0	101	367	8.7	176	.99	116	493	3.2	147	.99	116	453	7.1	112	.99	108	415	9.1	113	.99
	122	357	5.6	123	.99	101	487	3	114	.99	114	457	5.4	145	.99	104	421	7.8	123	.99
8.0	77	254	4.1	149	.99	74	291	10	170	.99	93	327	8.4	133	.99	79	255	7.7	114	.99
	84	262	5.3	134	.99	71	297	8	130	.99	84	321	9.6	127	.99	81	267	5.9	109	.99
10.0	48	172	4.8	103	.98	33	186	12	135	.98	56	160	12	129	.99	61	149	11	137	.99
	53	152	7.1	124	.98	46	180	14	127	.98	47	164	18	144	.98	56	144	9.8	112	.99

Units of Additional TAN concentration, P, R' and λ are g/L, ml CH_4, ml CH_4/g VSS/day and hr, respectively..

Table 8. Parameters determined from ATA with biomass acclimated to a TAN concentration of 5.77 g/L

Additional	pH = 6.5					pH = 7.0					pH = 7.5					pH = 8.0				
TAN conc.	P[a]	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2	P	R'	λ	SSE	R^2
Ctrl.	122	251	5.1	112	.99	124	401	5.8	142	.98	125	387	6.1	108	.99	123	201	3.2	107	1.0
2.0	108	274	6.1	107	.99	120	429	4.9	98	.99	128	405	4.3	116	.99	120	227	4.5	98	.99
	123	276	4.5	96	.99	118	431	4.8	110	.99	124	397	5.7	132	.98	125	218	4.4	129	.98
4.0	128	249	4.7	121	.99	127	412	5.4	57	.99	124	389	5.4	145	.98	112	184	8	148	.98
	120	245	5.1	114	.99	125	410	4.8	101	.99	125	387	6.1	104	.99	110	192	7.4	147	.98
6.0	98	122	9.1	114	.99	108	204	7.4	132	.98	110	258	8.1	132	.98	108	98	9.1	124	.91
	108	124	10	125	.99	106	198	8.1	142	.98	101	242	7.5	107	.98	104	97	7.8	109	.91
8.0	67	45	13	101	.84	104	142	9.2	174	.97	88	95	10	141	.98	79	31	7.7	133	.82
	77	53	15	142	.88	99	122	11	123	.98	98	107	9.8	162	.97	81	35	5.9	154	.81
10.0	58	25	17	132	.84	56	69	14	145	.90	46	67	15	124	.90					
	43	17	18	144	.76	54	58	15	162	.89	12	97	13	98	.90					

Units of Additional TAN concentration, P, R' and λ are g/L, ml CH_4, ml CH_4/g VSS/day and hr, respectively..

Ammonia Inhibition with Acclimation and pH effects

The results of quadratic regression of ammonia inhibition with predictors of TAN and pH are shown in Figures 5, 6, 7, 8, and 9, which illustrate the combined effect of pH, TAN, and acclimation on the specific methane production rate. In each of these figures, pH values are chosen between 5.5 and 8.5, and TAN concentrations from 0 to 13 g/L. The region is much wider than what was covered in the experiments. The peripheral area was predicted using the quadratic model for further comparison.

As mentioned earlier, ammonia inhibition is pH dependent (Eqs. 1 and 2). From the contours, it could be found that aceticlastic methanogens was active only within a short range of pH. At neutral pH, pH inhibition was not likely, but pH-dependent ammonia and ammonium species became the major parameters affecting the inhibition pattern. At pH values very much higher or lower than neutral, the pH effect became increasingly dominant and was the major cause of inhibition.

The differences among Figures 4, 5, 6, 7, and 8 at a pH could be regarded as the effect of acclimation. Acclimation can be described as a self-adjustment of methanogens that enabled them to survive the adverse conditions. By comparing the contours, it could be found that higher acclimation concentrations decreased the overall methanogenic activities. The peak SMAs were around 1.40, 1.40, 1.05, 0.76, and 0.63 L CH_4/g VSS/day for the five acclimation concentrations. Besides, an osmosis effect was observed, that the peak SMA for each kind acclimated biomass was always observed around its acclimation concentrations.

In addition, as acclimation concentration increased from 0.4 g/L to 5.77 g/L, an increase in the lethal concentration was observed. IC-100 (inhibitor concentration causing 100% inhibition) increased from 11 g/L for biomass acclimated to 0.40 g/L of TAN to 14 g/L for 5.77 g/L of TAN. Also, for acclimation concentration of 0.40 g/L, specific methanogenic activity decreased linearly as TAN increased. It was a kind of acute inhibition and methanogens could not tolerate the changes in pH, either. However, as acclimation concentration increased, the decrease in SMA as TAN increased started to show a short but fat shape. Methanogens could tolerate only a small change in pH and TAN. These results suggested that higher acclimation concentration increased the tolerance of methanogens in changing in pH, at the expense of decreasing SMA.

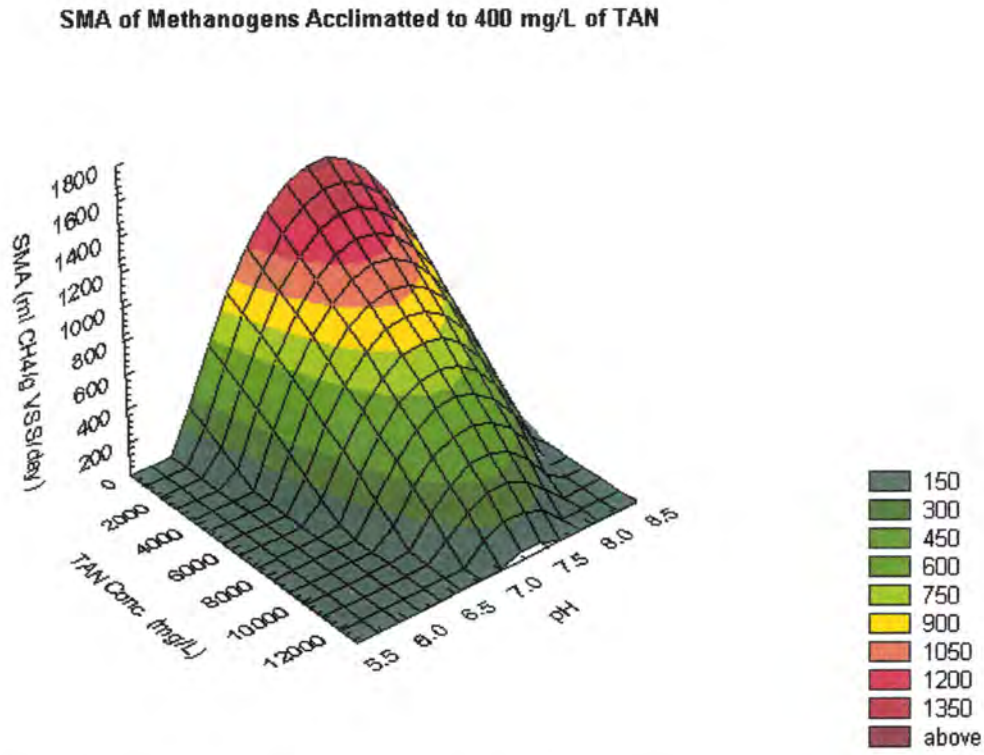

Figure 5. Specific Methanogenic Activity (SMA) determined from batch tests using quadratic regress for the biomass acclimated to TAN concentration of 0.40g/L

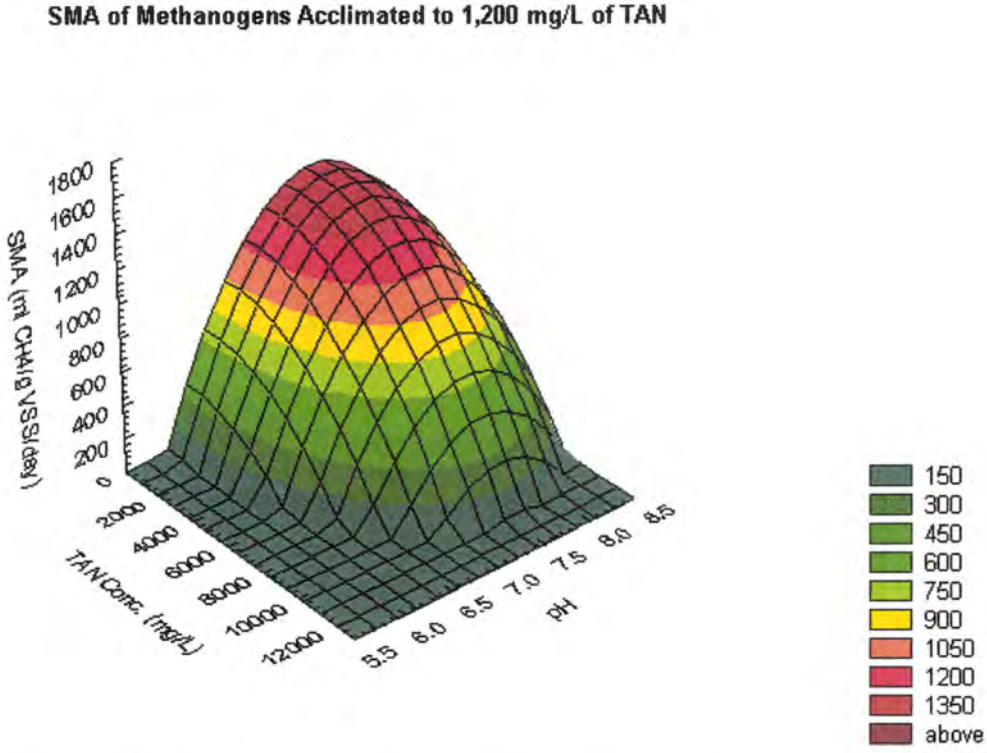

Figure 6. Specific Methanogenic Activity (SMA) determined from batch tests using quadratic regress for the biomass acclimated to TAN concentration of 1.20 g/L

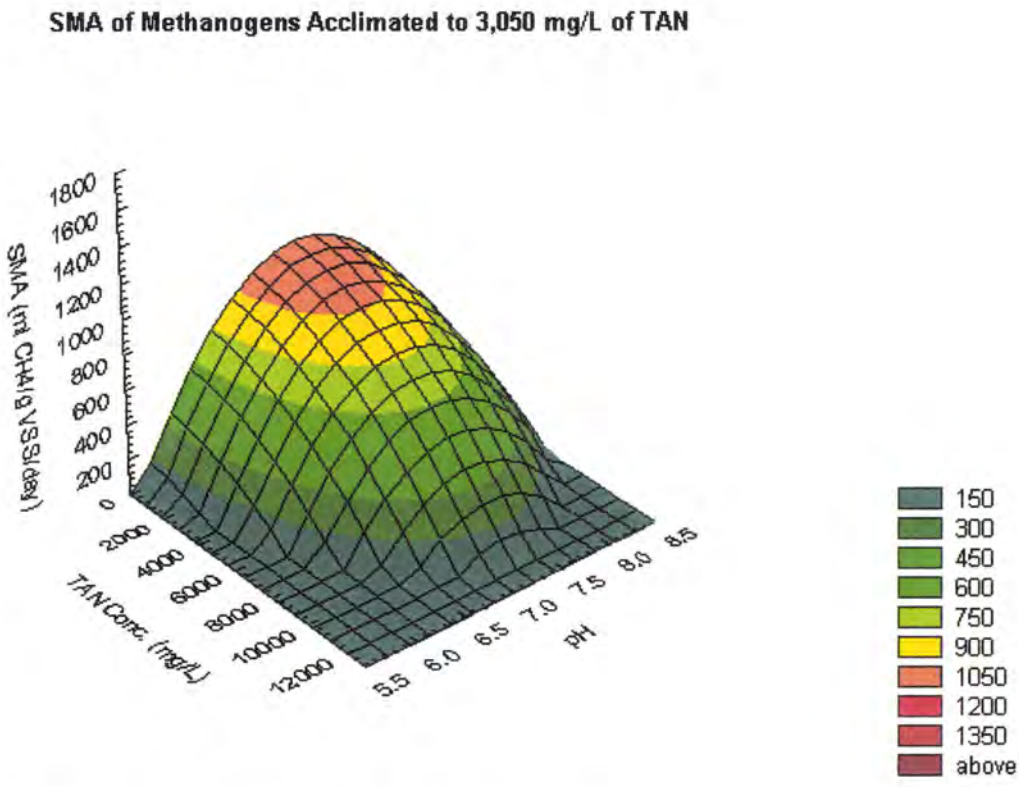

Figure 7. Specific Methanogenic Activity (SMA) determined from batch tests using quadratic regress for the biomass acclimated to TAN concentration of 3.05 g/L

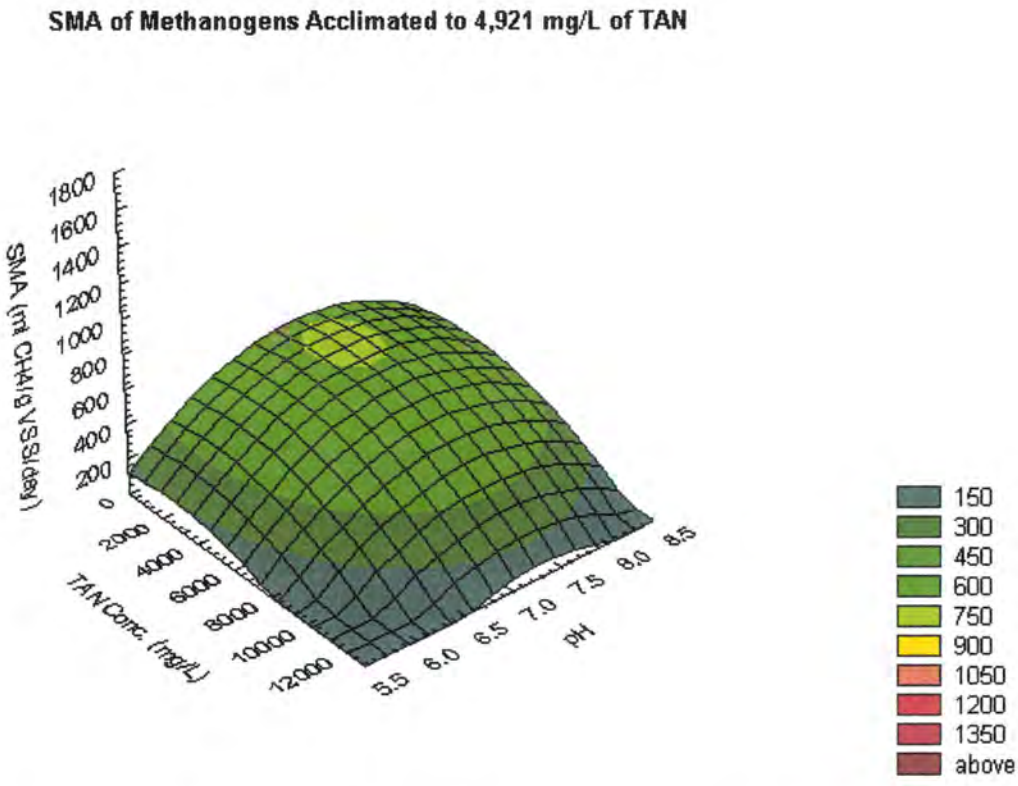

Figure 8. Specific Methanogenic Activity (SMA) determined from batch tests using quadratic regress for the biomass acclimated to TAN concentration of 4.92 g/L

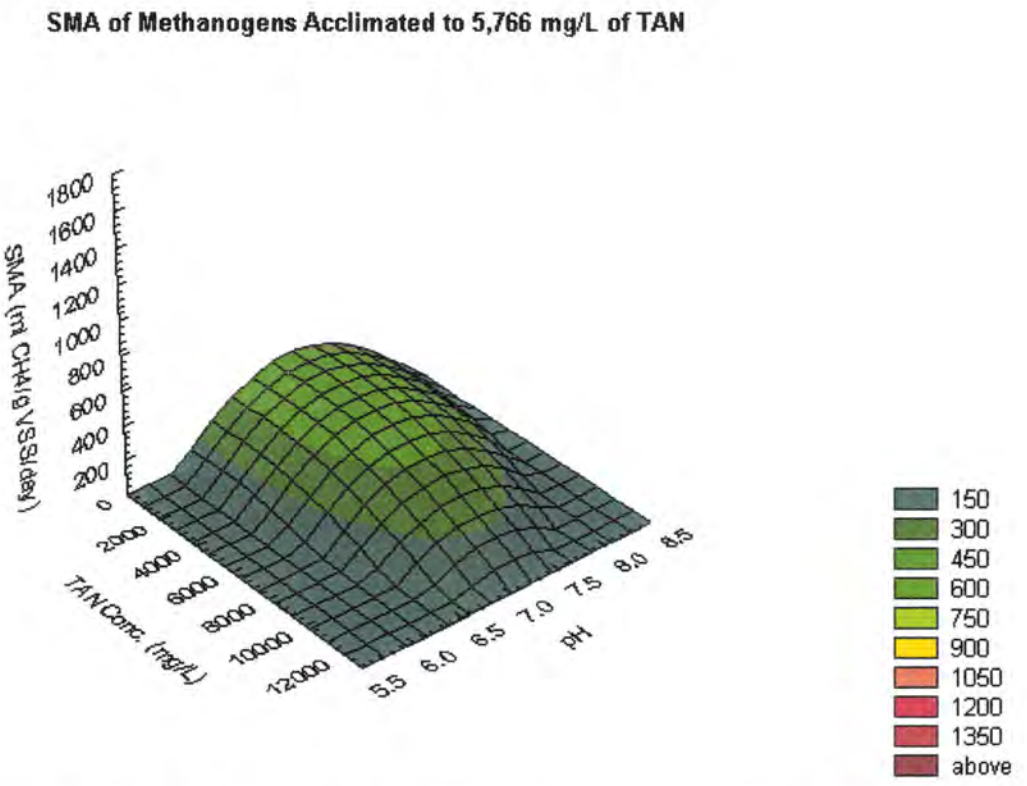

Figure 9. Specific Methanogenic Activity (SMA) determined from batch tests using quadratic regress for the biomass acclimated to TAN concentration of 5.77 g/L

CONCLUSIONS

It is apparent that the thermophilic aceticlastic methanogens are inhibited at high TAN concentrations. Based on the results of this research, the following conclusions are made:

1. The digester systems operated at an SRT of 7 days and a COD loading rate of 4 g /L/d, performed well when TAN concentrations in the reactor were increased to 1.2 g/L. The biogas production rate and pH were constant at around 18 L/d (68±3% CH_4 content) and 6.7 (55°C), respectively. No severe inhibition caused by ammonia was observed. However, TAN concentrations of 4.92 and 5.77 g/L caused the methane production rate to drop by 41 and 74 %, respectively.
2. Monod constants determined from batch tests with a background TAN concentration of approximately 0.40 g/L and pH of 7.3 were K_s=694.4 mg/L as COD and R_{max}= 1,927.4 ml CH_4/g VSS/day.
3. ATA results revealed that for acclimated biomass from the digester with a background TAN of 0.4 g/L, TAN concentrations higher than 2.00 g/L could cause inhibition. Biomass acclimated to 1.20 g/L as TAN showed comparatively higher activities when TAN was increased to 2.50 g/L. The lethal concentration was approximately 10.50 g/L as TAN throughout the tests with the acclimated biomass studied.
4. When acclimation concentration was increased (3.05, 4.92, and 5.77 g TAN/L), an overall decrease in SMA was observed at all pH levels tested between 6.5 and 8.0, but lethal concentrations increased to 10, 13, and 14 g/L, respectively. And, TAN

concentrations of 3.0, 4.0, and 6.0 g/L started to cause inhibition of these biomass.

5. At a constant TAN concentration, pH effect was significant for ammonia inhibition. The highest SMA was exhibited at pH range of 7.0 to 7.5. It was also observed that acclimation to high TAN concentration could increase methanogens' tolerance to changes in pH.

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CHAPTER 3. AMMONIA INHIBITION ON THERMOPHILIC ACETICLASTIC METHANOGENS

A paper to be submitted to the Journal of
Environmental Science and Technology

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ABSTRACT

The inhibition effects of ammonia, in terms of total ammonia nitrogen (TAN), under different pH values and acclimation conditions on thermophilic aceticlastic methanogens were investigated in this study. Completely-mixed anaerobic thermophilic digesters were operated at background TAN concentrations (acclimation concentrations) of 0.40, 1.20, 3.05, 4.92, and 5.77 g/L. The biomass acclimated to different TAN concentrations was collected from the digesters at steady state of each operation and further used in Anaerobic Toxicity Assays (ATA). ATA was the methodology used to measure the methanogenic activity in response to different pHs and TAN concentrations. The ATA were conducted at four different pH values (6.5, 7.0, 7.5, and 8.0) with TAN concentrations ranging up to 10.0 g/L. Extended Monod Equation was used to model the effect of TAN, while Normalized Michaelis Equation was used to model pH effect. Least square estimation was used to estimate the parameters in the two models. The results from ATA showed: 1) TAN concentrations higher than 4 g/L could cause obvious inhibition of aceticlastic methanogens. 2) After methanogens became acclimated to high TAN concentrations, the specific methanogenic activities (SMA) decreased. 3) Biomass

acclimated to higher TAN concentrations could alleviate the inhibition effect due to the increase in TAN concentration. 4) The lethal TAN concentration for methanogens was approximately 10 g/L, but high acclimation concentrations of 4.92 and 5.77 g/L could increase the lethal concentration to 12 and 15 g/L, respectively. 5) ATA results also revealed the role played by pH. At a given TAN concentration, methanogenic activity varied with the pH values. The highest methanogenic activity was always observed at a pH of 7.0~7.5. 6) It was also observed that acclimation could increase the pH tolerance range, which made the methanogens less sensitive to pH changes.

Keywords:

Anaerobic, thermophilic, ammonia inhibition, ATA, aceticlastic methanogenic activity, Extended Monod Model, Normalized Michaelis Model.

INTRODUCTION

Anaerobic processes operated under thermophilic conditions have gained attention due to its apparent advantages, such as high pathogen destruction, enhanced hydrolysis of complex biological materials, and reduction in foaming. It has been the technology of choice for sludge stabilization due to better volatile solids destruction, higher biogas production potential and, most importantly, the production of residual biosolids that meet US 40 CFR part 503 Class A biosolids specification.

However, anaerobic digestion operated at higher temperatures is sensitive to environmental factors such as pH, temperature, etc. It is in this context that the inhibition effect of ammonia on thermophilic anaerobic process becomes significant.

Ammonia is the main hydrolysis product formed by the degradation of organic proteineous material. Thermophilic anaerobic digesters used for the treatment of animal wastes such as swine manure, cattle and chicken wastes, and some of the industrial wastes such as food processing waste streams often encounter very high ammonia concentrations.

Ammonia is an essential nutrient for anaerobic microbes. Total ammonia nitrogen (TAN) concentrations of approximately 200 mg/L are believed to be beneficial to the anaerobic process. However, high concentrations of TAN can decrease microbial activities. Many studies (Van Velsen, 1979) have reported the toxic effect of ammonia at TAN concentrations of 2,000 mg/L. Acclimation is another phenomenon that needs to be evaluated. When methanogens become acclimated to ammonia, higher TAN levels can be tolerated. Mesophilic system studied by Parkin and Miller (1982) performed well at 9000 mg/L of TAN after acclimation.

In anaerobic systems, there exist equilibriums among ammonium ion ($NH_4^+{}_{(aq)}$), free ammonia ($NH_{3\,(aq)}$) in solution, ammonia ($NH_{3\,(g)}$) in gas phase, hydrogen ion (H^+) and hydroxyl ion (OH^-), as shown in Equations 1, 2 and 3:

$$NH_4^+{}_{(aq)} \Leftrightarrow NH_{3\,(aq)} + H^+ \qquad K_a = [NH_{3(g)}][H^+]/[\,NH_{3(aq)}] \qquad (1)$$

$$H_2O \Leftrightarrow H^+ + OH^- \qquad K_w = [H^+][OH^-] \qquad (2)$$

$$NH_{3\,(g)} \Leftrightarrow NH_{3\,(aq)} \qquad K_H = [NH_{3(aq)}]/[\,NH_{3(g)}] \qquad (3)$$

Where K_a, K_w, K_H = equilibrium constants.

Apparently, from Eqs. (1) and (2), the [ammonium]/[ammonia] ratio is pH dependent. Therefore, to study the ammonia inhibition, pH needs to be considered.

Since most previous studies on ammonia inhibition were conducted under

mesophilic conditions, to further promote the application of thermophilic digestion, more work needs to be done to evaluate the effect of ammonia on the process. In this paper, ammonia inhibition was studied under thermophilic conditions combined with pH and acclimation effects.

MATERIALS AND METHODS

Anaerobic Digesters

Two CSTRs fed with soluble non-fat dry milk as organic substrate and operated under thermophilic (55±1°C) conditions were used in this study. Both digesters were operated at a chemical oxygen demand (COD) loading rate of 0.40 g/L/day and a hydraulic retention time (HRT) of 7 days. Ammonium concentrations in the feed to the digesters were set to five levels during the five phases. During the first phase, the substrate fed to digester had a background TAN concentration of approximately 0.40g/L. In the following four phases, digesters were operated at four other levels of TAN conditions (1.20 g/L, 3.05 g/L, 4.92 g/L, and 5.77 g/L). The TAN level inside digesters in each phase was used as acclimation concentration for acclimation studies.

Batch experiments (ATA)

The acclimated biomass was collected from the digester after attainment of steady state (acclimation conditions) which was defined by constant daily biogas production, effluent volatile fatty acid (VFA) concentrations and operating pH values within 5% variation for 5 consecutive days in each reactor. Batch anaerobic toxicity assays (ATA)

were then conducted in 250 ml serum vials containing 100 ml of mixed liquor from the reactors. Acetic acid (HAc) was used in these batch tests as substrate to study aceticlastic methanogenic activity. The vials were sealed with butyl rubber stoppers and incubated in a shaker chamber set at 200 rpm and 55°C. At the beginning of each experiment, pH and TAN were controlled and each experiment was conducted in duplicates. Biogas production was measured every 4-12 hours. Cumulative methane production in standard air pressure was then calculated and specific methanogenic activity (SMA) was determined, which was used further as an indicator for inhibition effect.

Assay methods

Gompertz Model. The modified Gompertz equation has been proved statistically to be sufficient to describe the bacterial growth (Zwietering et. al., 1990) of *L. Plantarum*. The equation was employed in this study to describe the cumulative methane production curve in a batch experiment (ATA) based on the study of Lay et al. (1996), who related the bacterial growth to metabolic biogas production.

$$M = P \cdot \exp\{-\exp\left[\frac{R' \cdot e}{P}(\lambda - t) + 1\right]\} \qquad (4)$$

where

M = cumulative methane production;

λ = lag-phase time;

P = methane production potential;

R' = methane production rate for the batch test;

e = constant (2.71828).

The estimated values for R', maximum methane production in each batch was divided by the total amount of biomass to calculate the SMA. In ATAs for each acclimation condition, SMAs were determined for pH values at 6.5, 7.0, 7.5, and 8.0 and TAN concentrations ranging from 0 to 10 g/L. Further studies, TAN effects modeling and pH effects modeling, were based on the SMAs from these batch tests.

Extended Monod Equation. The effect of ammonium or ammonia on methanogens at a specific pH was described by using the extended Monod Equation.

$$R(I)' = R_m(1-\frac{I}{I^*})^n\left[\frac{S}{S+K_s(1-\frac{I}{I^*})^m}\right] \quad (5)$$

where

R(I)' = methane production rate observed at a pH and a substrate level;

R_m = maximum methane production rate;

S = substrate concentration;

K_s = half-saturation coefficient;

I = inhibitor concentration;

I^* = lethal ammonium or ammonia concentration;

n, m = coefficients.

The extended Monod equation includes three extra parameters (n, m, and I^*) to embody the inhibition effects. The method of Han and Levenspiel (1988) presented a good method to solve this based on conditional linear transformation, transforming the above non-linear model to linear form by treating some variables as fixed values. Functions of parameters would then be estimated given these fixed variables. In sequence, these functions of

parameters with the given variables were to be further transformed to other linear forms. The rest of the parameters could then be estimated by linear regression. Since transformations were made more than twice, it was difficult to estimate the error term associated with the original model. In addition, this method generally required relatively large samples/observations for good estimations. Lay et. al. (1998) proposed to use a simplified form of extended Monod equation, which only included two parameters for inhibition effect. The simplified equation was easier to use, since it could be transformed to a linear model if lethal inhibition concentration was given. However, this simplified form could lose some information about inhibition characteristics when an inhibitor is present in relatively low concentration. In this paper, Gauss-Newton procedure was used to perform least square estimation of the parameters (R_m, m, n, and I*) in the extended Monod equation. This method used Taylor expansion to approximate the non-linear model with a linear function of the parameters. Starting values for model parameters need to be assigned ahead, with a starting sum square of error calculated. Then, ordinary least square (OLS) estimation could be applied to the linear approximation to "update" estimates of the parameters. The iterations were continued until some convergence criterion was satisfied, e.g. $SSE^{(i-1)}$-$SSE^{(i)}$ < a constant. The estimates for parameters from this method would have a minimum sum square of errors.

Actually, the inhibition of TAN is confounded by several other factors such as ammonia/ammonium ratio, hydrogen ion, hydroxyl ion, etc. Due to the dependency among these factors, given a pH and for a specific acclimated biomass, TAN can be treated as a single inhibitor. Then, extended Monod Equation would be applicable to describe the

inhibition.

Parameters n and m in extended Monod Equation are generally used to determine the types of inhibition. Theoretically, m influences the inhibition pattern when inhibitor is at low concentrations while n determines the inhibition pattern at high concentrations of inhibitor. If both m and n are greater than zero, the inhibition is defined as uncompetitive inhibition (Grady *et. al.*, 1999)

Michaelis pH Function. The effect of pH on methanogenic activity at a given TAN concentration was analyzed by a Michaelis pH function (Angelidaki *et al.*, 1993), normalized to give a value of 1 as central value as shown in Eq. (6). Parameters, pK_l and pK_h, are lower pH dropoff value and higher pH dropoff value, respectively. (Dropoff is defined as the pH at which methanogenic activity is reduced by 50%)

$$F(pH) = R_0 \frac{1 + 2 \cdot 10^{0.5 \cdot (pK_l - pK_h)}}{1 + 10^{(pH - pK_h)} + 10^{(pK_l - pH)}} \qquad (6)$$

where

F(pH) = specific methanogenic activity at a particular pH with TAN concentration fixed;

pK_l = lower pH dropoff value;

pK_h = higher pH dropoff value;

R_0 = methane production rate at optimum pH.

The estimation of parameters in Michaelis pH function also used Gauss-Newton procedure for least square estimation described earlier. The estimated value, pK_h - pK_l, is actually the pH dropoff range, which can be defined as pH tolerance range (without losing the activity). A higher value would be desirable, since it would suggest that active

methanogens exist in a relatively wider range of pH (methanogens are robust toward the change in pH).

RESULTS AND DISCUSSIONS

Monod Kinetic Constants Determination

As the first part of the study, a series of batch tests were run at different substrate (acetic acid) concentrations (110.9 mg/L, 537.6 mg/L, 964.3 mg/L, 1,390.9 mg/L, and 1,817.6 mg/L as COD) to determine the baseline Monod constants. The biomass used was taken from the digester that was subjected to a background ammonia concentration of 0.40 g/L, which was solely from the substrate. It was assumed that at this condition, there was no ammonia inhibition. The cumulative methane production from each batch was recorded and a curve was fitted using Gompertz equation by least-square non-linear regression. The R' determined from Gompertz regression was used as specific methanogenic activity (SMA) after dividing by the volatile suspended solid (VSS). Fig. 1 shows the SMA at different substrate levels. Monod kinetic constants ($R=R_{max}(S/S+K_s)$) were subsequently determined using linear regression to be K_s=694.4 mg/L as COD and R_{max}= 1,927.4 ml CH_4/g VSS/day.

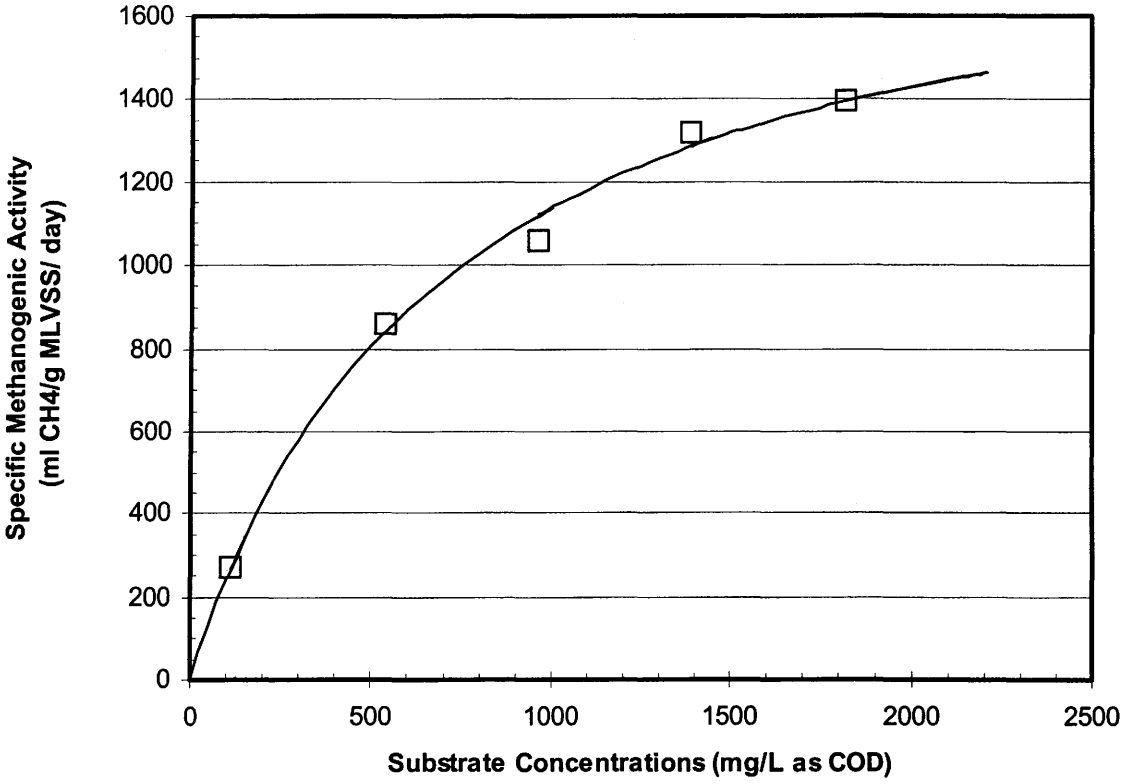

Figure 1. Specific methanogenic activities at various substrate levels. ("□ " Experimental data from batch tests; "———" predicted value using Monod equation)

Effect of TAN on Methanogenic Activity at Various pHs for different Acclimated Biomass

Acclimated biomass, obtained from steady-state anaerobic digesters operated at five acclimation concentrations of TAN, was used for ammonia toxicity study. The toxicity assays were conducted in serum vials with the pH adjusted to 6.5, 7.0, 7.5, and 8.0. Phosphate buffer solution was added to stabilize the pH. At each pH, the ammonia concentrations in serum vials were adjusted from background concentration up to 10 g/L as TAN by adding ammonium chloride.

In the ATA, biomass acclimated to ammonia concentrations of 0.40, 1.20, 3.05, 4.92, and 5.77 g/L as TAN. For each kind of acclimated biomass, a series of batch tests were run at various TAN and four pH values with SMA determined for each batch. Figures 2, 3, 4, 5, and 6 give the plots of SMA versus TAN at four pH values for each acclimated biomass. In each of these figures, there are four sets of data with each set standing for a series of batch runs at a pH.

Extended Monod Equation was used to study ammonia inhibition at a given pH. Non-linear least square regression function [nls( )] in S-plus statistic software package was performed to estimate the parameters, I*, R_m, n, and m in Eq. (5). The solid lines in the figures are predicted values using Eq. 5 with fitted parameters (I*, R_m, n, & m). For Fig. 4, the experimental points at highest TAN concentration were not included in the regression. Since, methanogens did not show activity at this TAN level, lethal TAN concentration was assumed to be below this TAN level.

The best-fit parameters are summarized in Table 1. It was noticed that n value at four pH values did not change greatly for each acclimated biomass, which suggests that n

had no clear correlation with pH. However, n seemed to be inversely related to the acclimation concentration. Higher acclimation concentrations lowered the parameter n and n became negative when acclimation concentration was increased to 5.77 g/L. On the other hand, parameter m increased as acclimation concentration increased to 3.05 g/L and then decreased with further increase in acclimation, showing a quadratic trend. Throughout the batch tests, n values were less than 1. When acclimation concentration was less than 4.92 g/L, ammonia inhibition showed uncompetitive inhibition characteristics ($n>0$ and $m>0$), and when acclimation concentration increased to 5.77 g/L ammonia inhibition switched to a undefined inhibition type ($m<0$ and $m<0$) (Grady *et al.*, 1999).

The results from the run with biomass acclimated to TAN concentration of 0.40 g/L show a rapid linear decrease in SMA with increasing TAN concentrations. For biomass acclimated to TAN concentrations of 1.20, 3.05, 4.92, and 5.77 g/L, the fitted curves show a convex trend. In this study, biomass acclimated to TAN concentrations of 0.40 g/L and 1.20 g/L showed peak SMA of approximately 1,400 mL CH_4/gVSS/day. However, for TAN concentration of 3.05 g/L, 4.92 g/L, and 5.77 g/L, the peak SMA dropped to 1,100, 650, and 450 mL CH_4/gVSS/day, respectively. The peak SMA values were always observed close to the acclimated TAN concentrations in the CSTR from where the biomass was taken for the ATA runs. The decreasing trend from the peak point of the curve to the Y-axis (TAN concentration = 0 mg/L) may be caused due to the effect of TAN osmotic pressure difference between acclimation and test concentrations. The acclimation effect could be observed by looking through Figs. 2, 3, and 4 sequentially. The fitted curves

became flatter and the difference among curves due to different pH became smaller. The methanogens after acclimation to higher levels of TAN became less sensitive to the changes of TAN and pH.

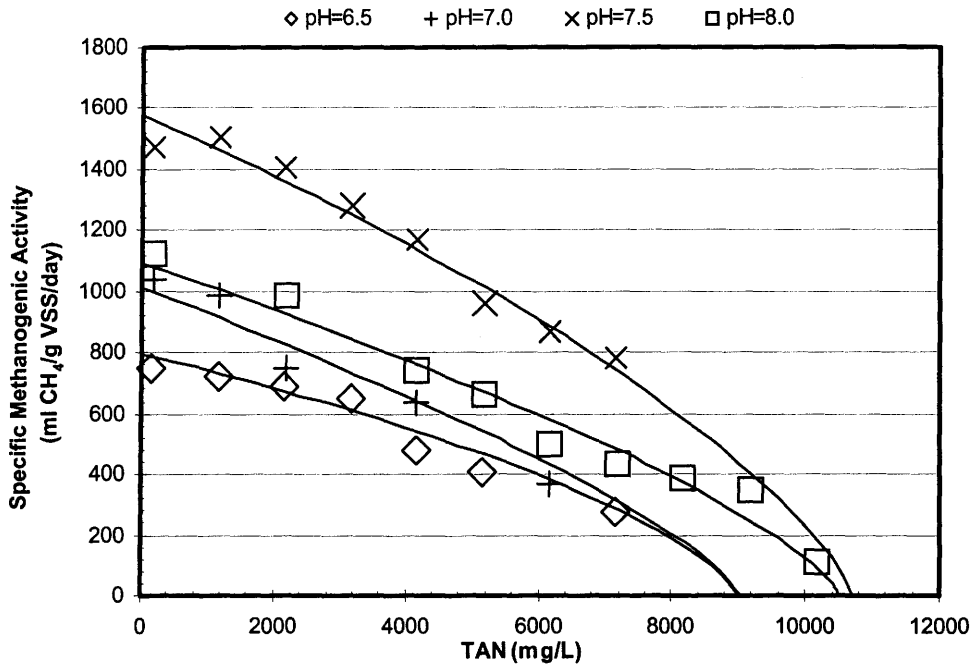

Figure 2. Relationship between SMA and TAN concentration at various levels of pH for biomass acclimated to 0.40 g/L of TAN

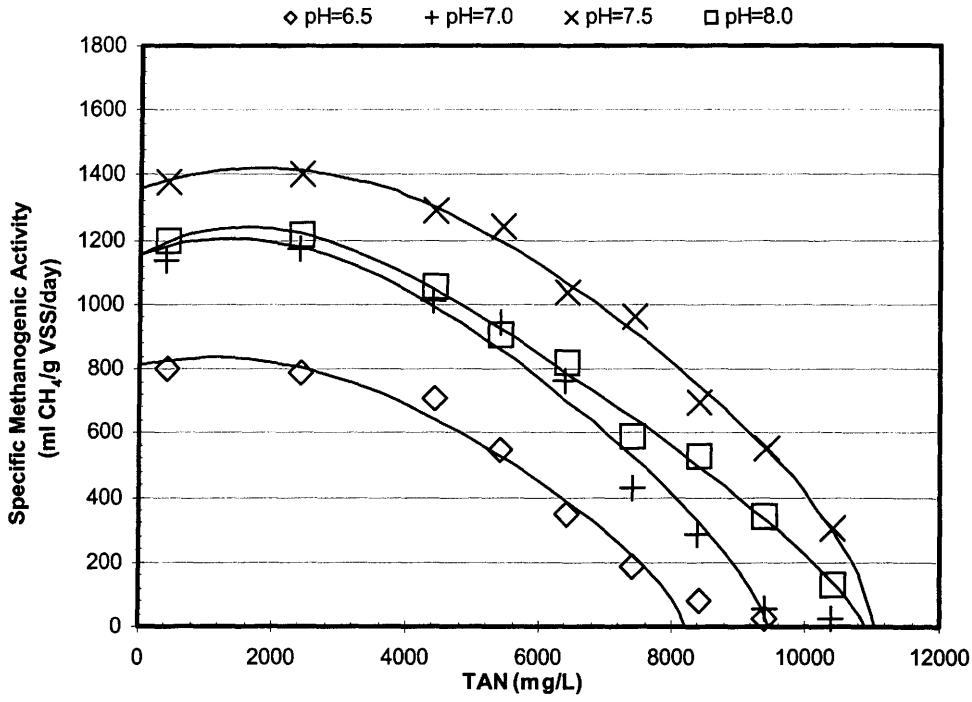

Figure3. Relationship between SMA and TAN concentration at various levels of pH for biomass acclimated to 1.20 g/L of TAN

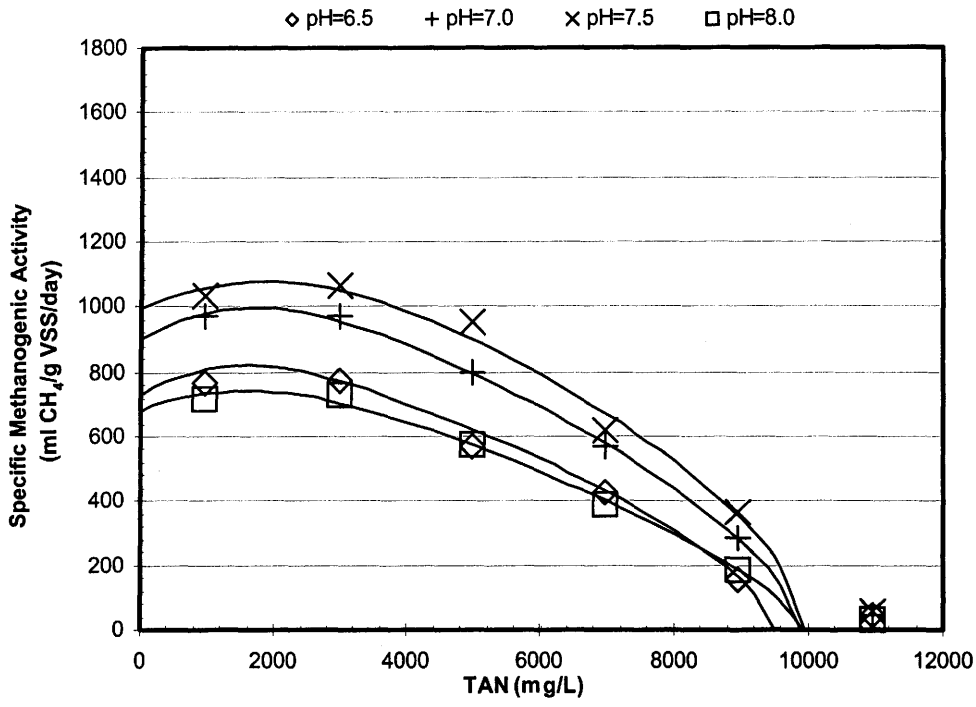

Figure 4. Relationship between SMA and TAN concentration at various levels of pH for biomass acclimated to 3.05 g/L of TAN

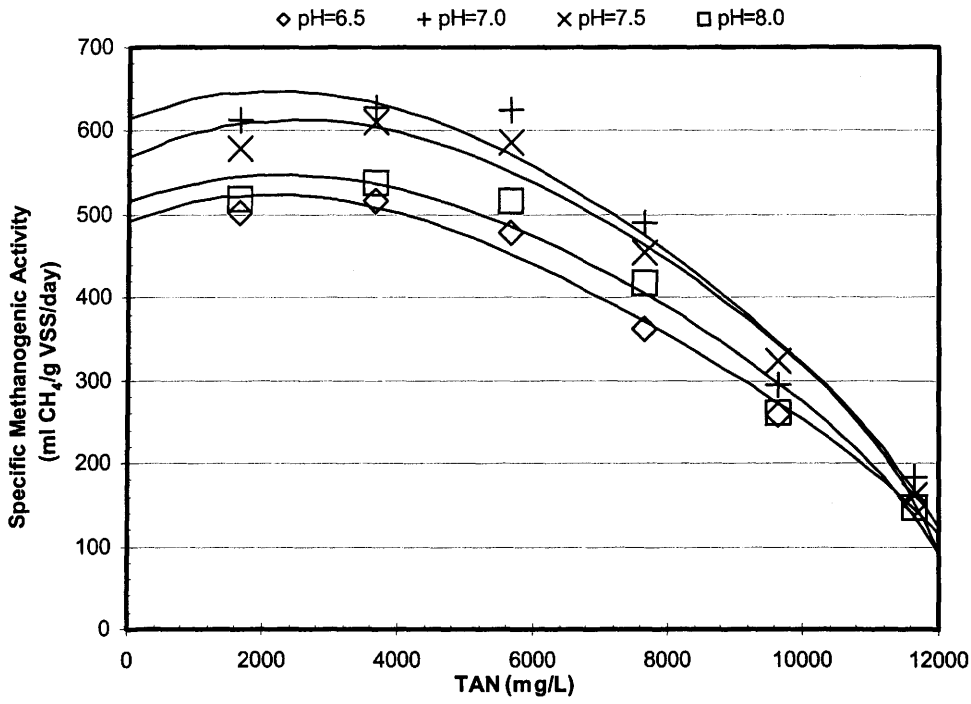

Figure 5. Relationship between SMA and TAN concentration at various levels of pH for biomass acclimated to 4.92 g/L of TAN

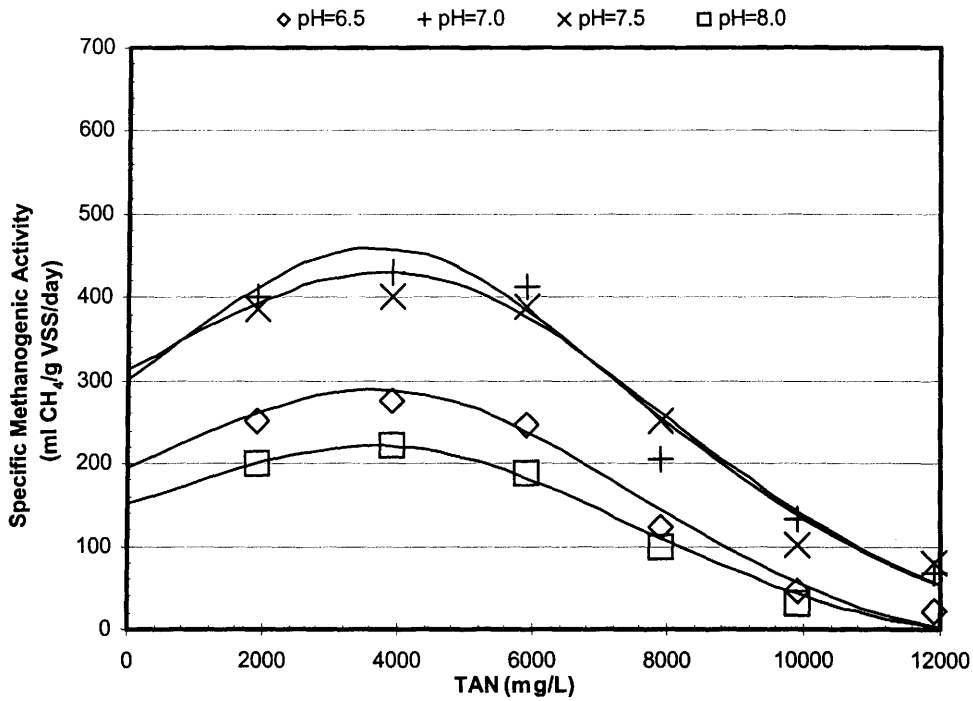

Figure 6. Relationship between SMA and TAN concentration at various levels of pH for biomass acclimated to 5.77 g/L of TAN

Table 1. Summary of the kinetic parameters of I^*, R_m, n, and m for the Extend Monod equation for the three acclimated biomass at various pH conditions

Acclimation Conc. (g/L as TAN)	pH	I* (mg/L)	R_m ($mlCH_4/gVSS/d$)	n	m	SSE	r^2
0.40	6.5	9000	1100	0.762	0.605	1.12E+04	0.921
	7.0	9050	1400	0.784	0.150	2.07E+06	0.966
	7.5	10700	2180	0.786	0.461	2.51E+06	0.967
	8.0	10500	1510	0.750	0.132	2.45E+04	0.958
1.20	6.5	8200	1123	0.698	4.007	8.13E+03	0.977
	7.0	9450	1598	0.723	4.632	2.48E+04	0.968
	7.5	11037	1879	0.640	4.271	8.22E+03	0.993
	8.0	10902	1592	0.790	6.805	6.15E+03	0.992
3.05	6.5	9495	995.1	0.631	8.233	4.88E+03	0.993
	7.0	9880	1235	0.622	6.739	4.29E+02	0.999
	7.5	9956	1359	0.585	5.424	6.99E+03	0.981
	8.0	9928	933.2	0.695	6.680	1.17E+03	0.995

Table 1. (continued)

Acclimation Concentration (mg/L as TAN)	pH	I* (mg/L)	R_m ($mlCH_4/gVSS/d$)	n	m	SSE	r^2
4,921	6.5	12890	671.8	0.655	5.37	1.40E+03	0.974
	7.0	12492	840.6	0.597	4.39	6.95E+03	0.927
	7.5	12219	778.0	0.525	4.70	2.65E+03	0.958
	8.0	12352	706.2	0.570	4.406	3.12E+03	0.949
5,766	6.5	12367	268.6	-3.515	-5.103	8.48E+02	0.989
	7.0	15390	410.6	-4.956	-6.984	4.30E+03	0.956
	7.5	15204	428.7	-3.651	-5.639	2.82E+03	0.982
	8.0	12352	206.0	-3.515	-5.103	2.73E+02	0.998

[a]SSE and r^2 are sum of square error and R-square, respectively

Figure 7 illustrates the theoretical (R_m) and experimentally observed (peak SMA) SMAs for different acclimated biomass. R_m refers to the theoretical maximum SMAs predicted from the extended Monod equation (Eq. 5) under conditions of infinite substrate concentration and in the absence of the inhibitor. Peak SMA values, as reproduced on Figure 7 from Figs 2-6, are the corresponding experimental SMAs obtained from ATAs in presence of the inhibitor and under non-limiting conditions of substrate. Comparison of R_m and peak SMA values for each acclimated biomass indicates a notable difference. And, the difference between R_m and peak SMA diminishes at higher acclimation concentration minimizing the loss of SMA, observed at lower concentrations. The peak SMA could approach, but never exceed R_m, under identically optimum conditions.

However, there is a benefit associated with acclimation. Figure 8 gives the IC50 and IC100 values determined for each kind of acclimated biomass. IC50 and IC100 are TAN concentrations that can decrease SMA by 50 and 100%, respectively. There are increasing trends in both IC50 and IC100 with higher acclimation concentration. The increase in IC100 implies methanogens acclimated at higher TAN concentration can survive a higher load of TAN. While, increase in IC50 suggest the decreased sensitivity of methanogens to TAN concentration.

As can be seen in Figures 2-6, after acclimated to a higher TAN concentration, methanogens always showed the highest activity around acclimation concentration. This can be explained by osmosis pressure formed inside methanogens after acclimation. A sudden drop in TAN concentration in circumstance could create acute toxicity to methanogens, since water molecules will more easily permeate into the methanogen cell

and interrupt metabolic balance. Figure 9 shows that relationship between acclimation concentrations and these optimum TAN concentrations at peak SMAs. The optimum TAN concentrations were no longer close to zero when methanogens were acclimated to higher TANs. However, they were not exactly the same as acclimation concentration (shown as dash line in Figure 9). The optimum TAN concentrations were actually falling below acclimation concentration.

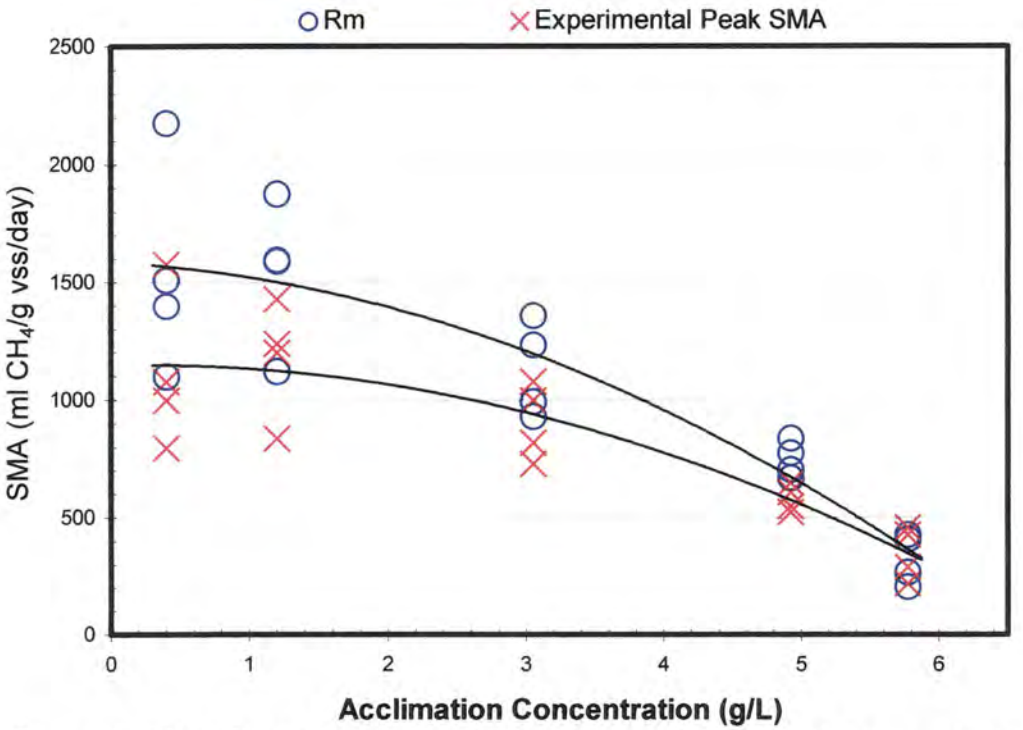

Figure 7. The effect of acclimation on the R_m, maximum SMA in extended Monod model, and peak SMA observed in ATA

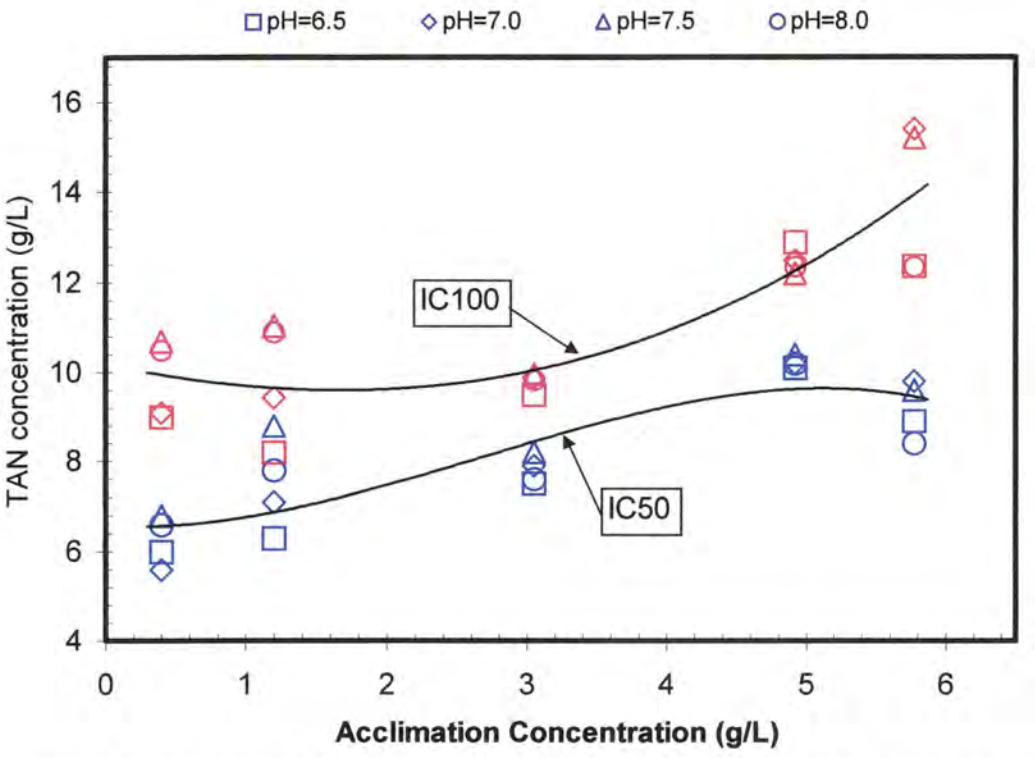

Figure 8. IC50 and IC100 observed in ATA for five kinds of acclimated biomass (Red, IC100; Blue IC50)

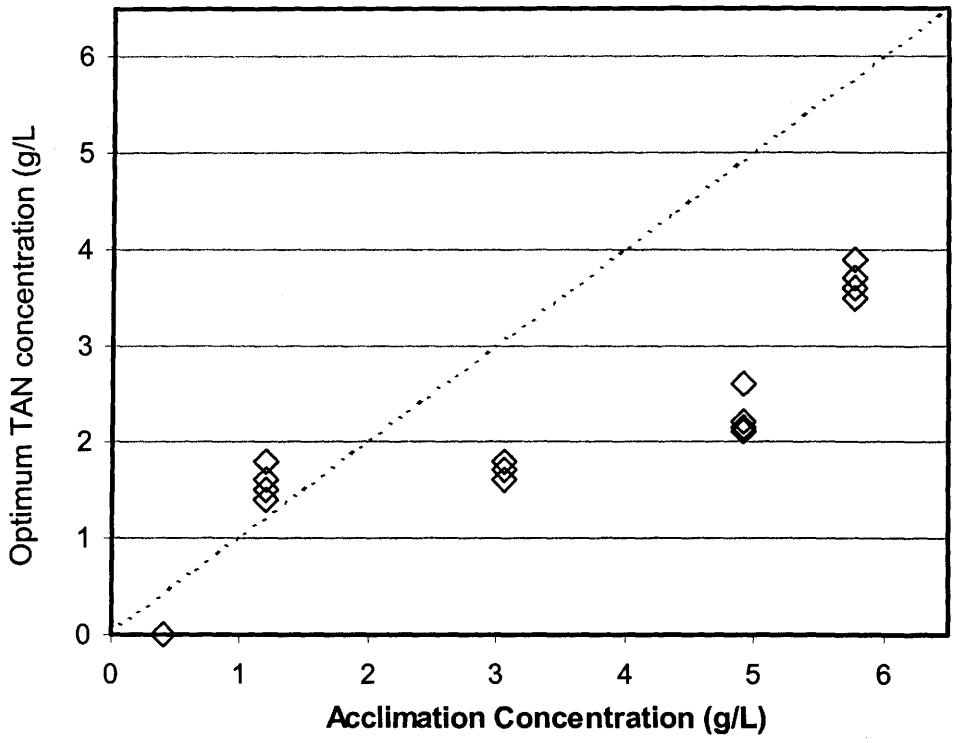

Figure 9. The relationship between optimum TAN concentration and acclimation concentration in ATA

Effect of pH in Ammonia inhibition

To demonstrate the role of pH in ammonia inhibition, SMAs at various pH values for the five kinds of biomass at given TAN concentrations are plotted in Figs. 7, 8, 9, 10, and 11. Normalized Michaelis pH function (Eq. 6) was used to model the pH effect. The nls( ) function in the S-plus software was used to perform non-linear regression and the solid lines in the figures show the predicted values using the fitted parameters. The best-fit values of the kinetic parameters are summarized in Table 2. Figs. 7, 8, 9, 10, and 11 indicate that aceticlastic methanogens show activity only within a narrow pH range between 5.5 to 8.5 and can be inhibited by high TAN concentrations. After acclimation, though there was a decrease in the peak SMA, the curves become flatter suggesting that the methanogens could operate over a wider range of TAN concentrations and pH.

As can be seen from the above figures and table, the Michaelis Model predicts the pH effect well in the sense that pH effect can also be understood as another inhibition. The highest activity was observed over a narrow range of pH of 7.0~7.5 from the batch tests for the acclimated biomass.

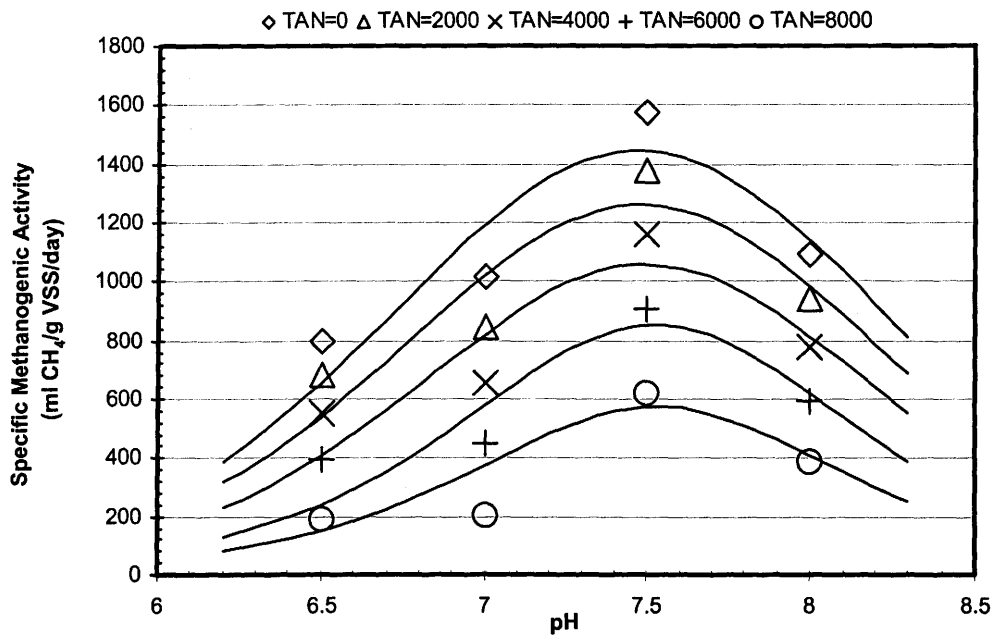

Figure 10. Relationship between SMA and pH concentration at various levels of TAN for biomass acclimated to 0.40 g/L of TAN

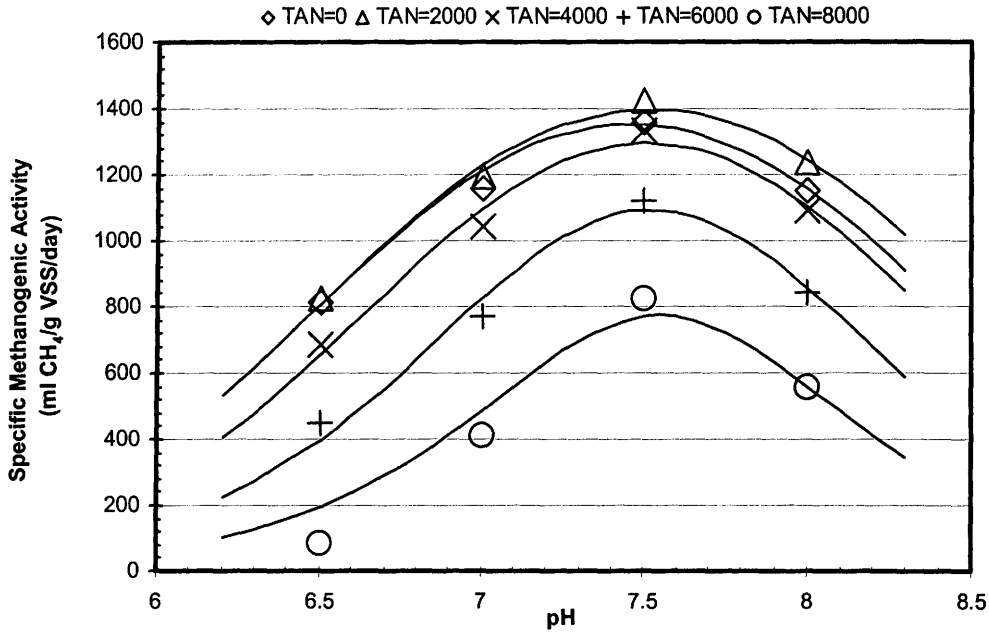

Figure 11. Relationship between SMA and pH concentration at various levels of TAN for biomass acclimated to 1.20 g/L of TAN

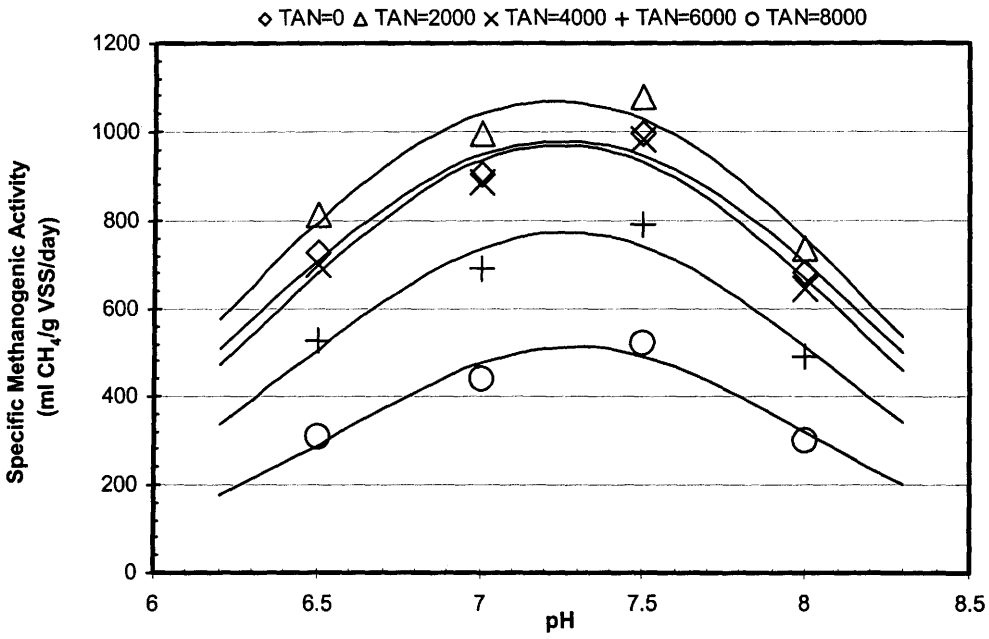

Figure 12. Relationship between SMA and pH concentration at various levels of TAN for biomass acclimated to 3.05 g/L of TAN

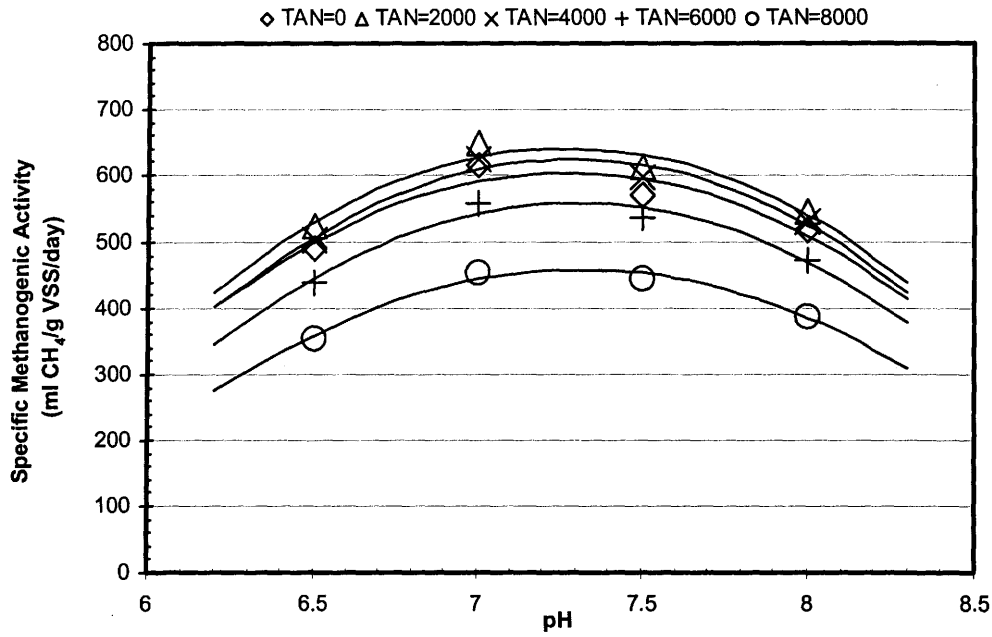

Figure 13. Relationship between SMA and pH concentration at various levels of TAN for biomass acclimated to 4.92g/L of TAN

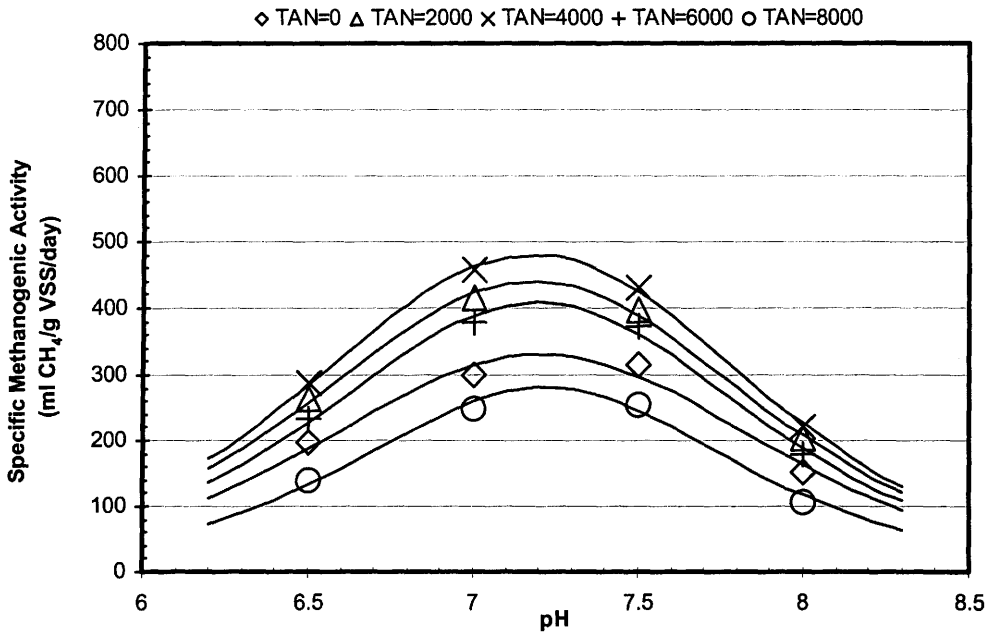

Figure 14. Relationship between SMA and pH concentration at various levels of TAN for biomass acclimated to 5.77 g/L of TAN

Table 2. Summary of the kinetic parameters of pK_l, pK_h, and R_0 for the Normalized Michaelis pH function for the three acclimated biomass at various TAN concentrations. (SSE and r^2 are sum of square error and R-square, respectively).

Acclimation Concentration (mg/L as TAN)	TAN (mg/L)	pK_l	pK_h	R_0	SSE	r^2
400	0	6.85	8.10	1445	7.26E+04	0.791
	2000	6.90	8.05	1260	6.80E+04	0.762
	4000	7.02	7.97	1059	5.93E+04	0.744
	6000	7.36	7.69	854.0	4.36E+04	0.777
	8000	7.46	7.60	574.0	3.28E+04	0.753
1,200	0	6.53	8.39	1351	3.31E+03	0.982
	2000	6.54	8.49	1396	2.20E+03	0.989
	4000	6.70	8.30	1295	5.81E+03	0.974
	6000	7.06	7.98	1093	6.45E+03	0.975
	8000	7.50	7.60	770.1	2.03E+04	0.994
3,050	0	6.35	8.14	980.2	5.15E+03	0.920
	2000	6.31	8.13	1069	5.42E+03	0.926
	4000	6.42	8.06	970.7	6.48E+03	0.913
	6000	6.53	7.98	774.6	5.74E+03	0.902
	8000	6.75	7.82	514.0	3.09E+03	0.913

Table2. (continued)

Acclimation Concentration (mg/L as TAN)	TAN (mg/L)	pK_l	pK_h	R_0	SSE	r^2
4,921	0	6.02	8.51	602.9	1.31E+03	0.856
	2000	6.03	8.51	640.7	8.31E+02	0.915
	4000	6.07	8.50	623.1	6.13E+02	0.938
	6000	6.11	8.48	558.3	4.23E+02	0.953
	8000	6.15	8.46	459.0	2.04E+02	0.969
5,766	0	6.85	7.56	331.7	6.85E+02	0.964
	2000	6.82	7.54	440.3	1.67E+02	0.995
	4000	6.82	7.53	480.6	1.08E+02	0.997
	6000	6.91	7.48	409.0	3.57E+02	0.988
	8000	7.21	7.23	281.2	3.57E+02	0.980

Figure 15 shows that relationship between acclimation and pH dropoff range, which is pH value between pK_l and pK_h. Similar to IC50 defined earlier, pH dropoff range is the range that pH will not cause inhibition by more than 50%. As shown in Figure 15, acclimation to higher TAN concentrations other than 5.77 g/L could increase the dropoff ranges. This suggests that acclimation (less than 4.92 g/L of TAN) could increase methanogens' ability to tolerate pH changes. Since, pH effect is significant in anaerobic digestion, acclimation to a certain level of TAN would be beneficial to a stable system performance.

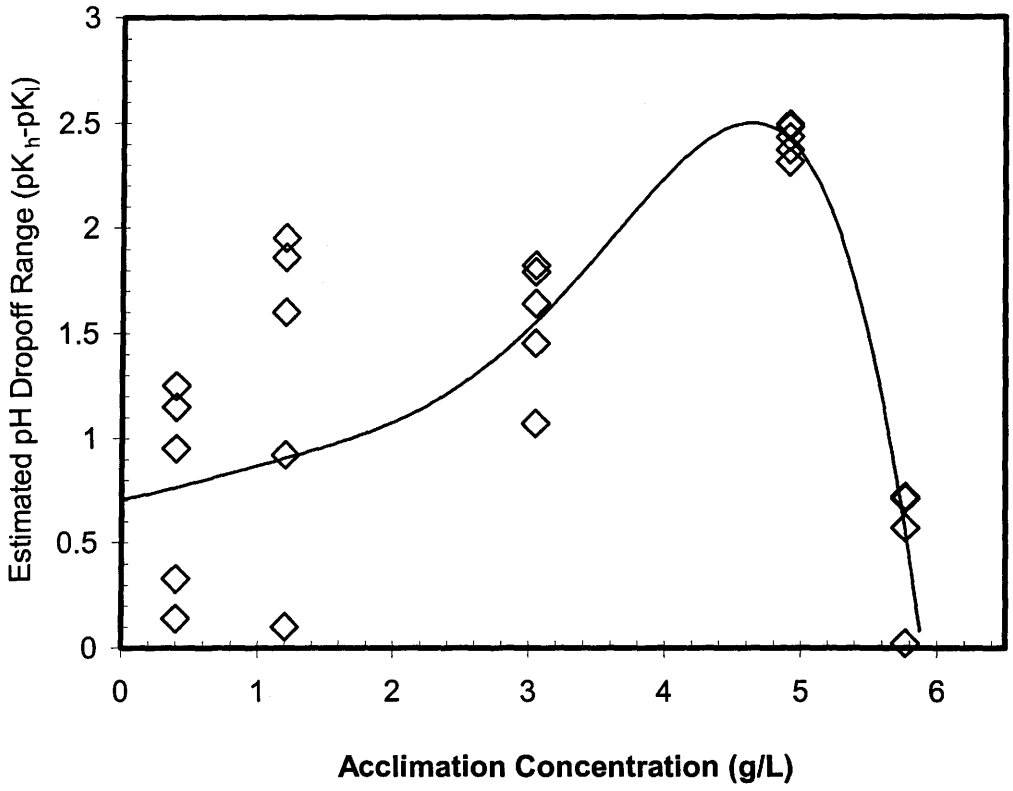

Figure 15. The effect of acclimation on pH dropoff range

CONCLUSIONS

Based on the results of this research, the following conclusions were made:

1. Monod constants at no ammonia inhibition condition were determined from batch tests at TAN concentration approximately 130 mg/L and pH 7.3. For this study, K_s = 694.4 mg/L as COD and R_{max} = 1,927.4 ml CH_4/g VSS/day.
2. ATA tests revealed that high TAN concentrations could cause inhibition. For biomass acclimated to 0.40 g/L as TAN, SMA decreased sharply as TAN concentration increased. Higher acclimation concentrations showed a lower rate of decrease of SMA. Acclimation could alleviate the TAN inhibition effect. The lethal TAN concentrations were around 10 g/L as TAN, while higher acclimation concentrations could increase the lethal concentration slightly.
3. For TAN inhibition, pH effect was significant. Methanogens showed activity only within a narrow range of pH and SMA varied with pH changes. The highest SMA was reported at pH = 7.0~7.5.
4. Extended Monod model and Normalized Michaelis pH function worked well in fitting TAN inhibition and pH effect, respectively. The best fitted parameters from Extended Monod model showed that ammonia inhibition followed uncompetitive characteristics, while high TAN acclimation concentrations of 5.77 g/L switch the inhibition to an undefined type. The fitted parameters from Normalized Michaelis model showed that high TAN concentrations would

narrow the pH dropoff range (pK_h-pK_l), while higher TAN acclimation concentration (≤4.92 g/L) could widen the dropoff range.

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CHAPTER 4. GENERAL CONCLUSIONS

GENERAL DISCUSSIONS

In this study, continuously operated anaerobic digester was operated at a SRT of 7 days and an organic loading rate of 4 g/L/d as COD. TAN concentrations less than 1.2 g/L inside digester did not cause obvious inhibition on methanogenesis. TAN concentrations of 4.92 and 5.77 g/L caused the methane production rate to drop by 41 and 74%, respectively.

Five kinds of acclimated biomass were used in ATA. They were collected from anaerobic digesters and were acclimated to different concentrations of TAN. The results of ATA showed that:

1. The effect of pH was significant in ammonia inhibition. It interacted with ammonia inhibition by changing the ratio of ammonia to ammonium and could inhibit methanogenesis dominantly at both high pH and low pH. The optimum pH for methanogenesis was between 7.0 and 7.5.
2. Acclimation also had an effect on methanogenesis. The optimum methanogenic activity of biomass acclimated to lower TAN concentration were usually higher than that of biomass acclimated to higher TAN concentration.
3. However, acclimation concentration could increase lethal concentration of methanogens and increase the tolerance of methanogens to the pH by increase the pH dropoff range.

RECOMMENDATION FOR FUTURE RESEARCHES

1. Compared with previous results obtained from mesophilic condition (De Baere, L. A., et. al., 1984; Hensen, K. H., et. al., 1998), ammonia was not as toxic to methanogens in thermophilic conditions as those in mesophilic conditions. A further study could be carried out to compare and investigate the differences in ammonia inhibition between the two conditions.
2. The investigation of ammonia inhibition in this paper considered two effect, pH and acclimation, beside the TAN concentration. The TAN could be treated as main effect, while pH and acclimation be treated as block effects. A further work could be executed with a better experimental design to investigate the ammonia inhibition by blocking pH and acclimation.

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